Factors affecting growth disparity in spiny lobster aquaculture: the effect of physiology, behaviour and feeding

by

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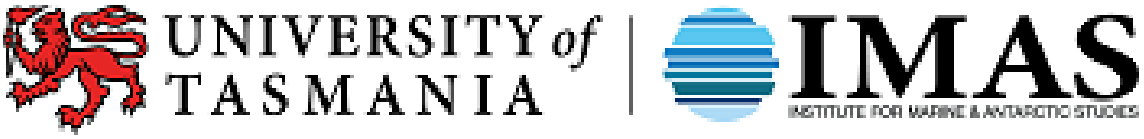

STATEMENTS AND DECLARATIONS

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STATEMENTS OF CO-AUTHORSHIP

Chapter 2-5 of this thesis have been prepared as scientific manuscripts and will be submitted for review in the near future. In all cases, the experimental design, data analysis and interpretation, and manuscript preparation were the primary responsibility of the candidate. Nevertheless, these studies were carried out in the collaboration with supervisors and co-authors. The contributions of co-authors are outlined below:

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GENERAL ABSTRACT

Spiny lobsters are known as a valuable commercial species with limited availability, which makes them a promising candidate for aquaculture. However, to date, the production of spiny lobsters in captivity has been characterised by considerable variation in individual lobster growth rates leading to growth disparity and impacting on biomass production. One explanation for the growth disparity in spiny lobsters is the agonistic behaviour of dominant individuals, whereby they control and consume a disproportionate share of food resources, benefiting their own growth performance. However, the mechanisms of how behavioural and feeding traits of individual lobsters influence growth disparity in culture are poorly understood. Furthermore, research of a range of marine organisms has shown that variability in individual metabolic physiology (metabolic phenotype) can be an important factor influencing behaviour and growth. However, the relationship between metabolic phenotype and individual growth performance has not been previously investigated in any spiny lobster species. Understanding the influence of intraspecific diversity in physiological traits on growth performance of individuals is an important consideration for the development of optimal rearing conditions and management strategies for spiny lobsters aquaculture. This is the first study to focus on the influence of individual variations in physiology, behaviour and feeding on the growth performance of two commercial temperate spiny lobsters species, *Sagmariasus verreauxi* and *Jasus edwardsii* juveniles in captivity.

In Chapter 2, the influence of metabolic phenotype and social behaviour on growth performance of early juvenile *S. verreauxi* (5.99±2.77g) that were reared either individually or communally was examined. Findings show that communally reared lobsters have greater growth performance, survival and feed intake indicating that social interaction is vital for promoting the growth of lobsters. Growth performance of individually reared lobsters was positively linked with metabolic rate providing the first evidence of a link between metabolic phenotype and growth performance of a lobster species. Metabolic phenotype was not linked to lobster growth performance in communal culture indicating that social interaction outweighed the direct link between metabolic rate and lobster growth. These results suggest social behaviour plays a dominant role in determining the growth of individuals in populations, however, the factors influencing behavioural interactions between individuals within a population remained to be determined. In Chapter 3, the effect of metabolic phenotype, body size, sex, feeding contest experience and rearing history on the early juvenile *S. verreauxi* social status was examined using pair-feeding contest behavioural studies. Findings from these experiments showed that larger size lobsters were likely to be more dominant over smaller

lobsters. Low metabolic rate lobsters also displayed greater ability to win over high metabolic rate lobsters which may explain why growth was not positively linked with metabolic phenotype in communal culture, as demonstrated in Chapter 2. Female lobsters were more dominant than male lobsters irrespective of size and metabolic phenotype status. These findings showed that the dominance behaviour of *S. verreauxi* is complex and that a range of factors including body size, metabolic status and sex can influence dominance status and potentially growth of individual lobsters in captivity.

Chapter 4 examined the influence of emergent juvenile intraspecific size variation and metabolic phenotype on growth performance of *J. edwardsii* post-pueruli in individual or communal rearing. Similar to findings with *S.verreauxi*, communally reared *J. edwardsii* displayed a greater carapace length increment and a shorter intermoult period indicating that social interaction promotes lobster growth performance. Emergent juvenile body size did not influence lobster growth performance. Also similar to findings with *S. verreauxi,* metabolic phenotype showed a positive correlation with growth in individually reared lobsters, however, the communally reared lobsters displayed no such relationship suggesting that metabolic phenotype is also an important factor for *J. edwardsii* growth in the absence of social interaction. However, the underlying mechanism contributing to the relationship between metabolic phenotype and growth of spiny lobsters were not well understood. Chapter 5 examined the effect of metabolic phenotype on individual variation in feed intake and food preference between current best diets of the emergent juveniles *J. edwardsii* and linked with individual growth performance. Metabolic phenotype was not related to individual lobster feed intake. Moreover, lobster growth performance was also not linked with their feed intake. Mussel, notably mussel gonad, was the most preferred food for *J. edwardsii* emergent juveniles. Lobster food preference showed no correlation with the individual growth performance. These findings indicated that lobster feed intake and preference are not fundamental factors linking metabolic phenotype and growth.

Collectively, findings from my study show that growth of spiny lobsters in culture is highly complex and influenced by a range of intrinsic and extrinsic factors. The present study provided the first evidence that spiny lobsters growth can be directly linked to metabolic phenotype, however, social behaviour appears to play a greater role in promoting the growth of individuals and populations. The dominance status of spiny lobster during feeding competition can be influenced by body size, metabolic phenotype and sex. Thus, growth performance of spiny lobsters in captivity can be explained by a complex interaction between individual lobster physiological traits and social interaction. Emergent juvenile body size and

feed intake and preference is not a major driver for growth disparity in culture. The development of aquaculture systems for spiny lobsters may involve a trade-off between systems which promote overall growth at the expense of increases in growth disparity between individuals. Further research is required to determine the mechanism (visual, chemical and or physical) involved in the relationship between social interaction and growth of spiny lobsters in captivity.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Overview

Spiny lobsters are valuable and in high demand in international and local seafood markets (Skirtun et al., 2013 ; Francis et al., 2014) but with limited availability due to full exploitation of wild stock (Jones & Shanks, 2008 ; Kenway et al., 2009 ; Linnane et al., 2010 ; Ehrhardt & Fitchett, 2010 ; Phillips & Matsuda, 2011). Present commercial lobster aquaculture, mainly in Vietnam, depends on the capture of wild juvenile seed stock for on-growing to market size (Hung & Tuan, 2008). However, recent developments in spiny lobster hatchery technologies, particularly in Australia (Fitzgibbon & Battaglene, 2012a; Fitzgibbon & Battaglene, 2012b; Jensen et al., 2013a, Jensen et al., 2013b; Fitzgibbon et al., 2017), suggests that closed cycle aquaculture may soon be a reality. To meet both the global demand and to reduce the fishing pressure on wild stocks, the development of spiny lobster aquaculture requires advances in juvenile lobster processes to improve commercial and production efficiencies (Kittaka et al., 1997 ; Williams, 2009 ; Jones, 2010 ; Phillips & Matsuda, 2011).

Long-term feasibility and sustainability of spiny lobster aquaculture depends on closing the life cycle and developing an economically viable method for raising lobsters from eggs through to market size (Sachlikidis et al., 2005). To date, the production of spiny lobsters in captivity has been characterised by significant variation in individual growth rates leading to growth disparity and impacting on biomass production and uniformity of production in aquaculture (Irvin & Williams, 2008 ; Vijayakumaran et al., 2010 ; Carter et al., 2014). Earlier studies demonstrated that growth variability in spiny lobster culture can be affected by numerous abiotic and biotic factors (Travis, 1954 ; Phillips et al., 1992 ; Crear et al., 2000 ; Smith & Ritar, 2006 ; Montgomery et al., 2009 ; Vijayakumaran et al., 2010 ; Jensen et al., 2013b ; Fitzgibbon et al., 2017 ; Ratunil Jr, 2017). Metabolic physiology is a biotic factor that may influence growth of spiny lobster in captivity but, to my knowledge, has never been studied. Previous studies on teleosts have demonstrated that variability in individual metabolic physiology (metabolic phenotype) can be an important factor influencing an individual's growth as well as behaviour and feeding traits (Metcalfe et al., 1995 ; Yamamoto et al., 1998 ; Brown et al., 2003 ; Biro & Stamps, 2010 ; Auer et al., 2015a).

This thesis provides the fundamental knowledge in understanding the influence of intraspecific diversity in physiological traits, behaviour and feeding on growth performance of two commercial temperate spiny lobsters species, *Sagmariasus verreauxi* and *Jasus edwardsii* juveniles in captivity (Crear et al., 2000 ; Jeffs, 2010 ; Kittaka et al., 1997 ; Booth, 2006) . Recent success in the development of spiny lobster propagation technologies at the Institute for Marine and Antarctic Studies (IMAS), University of Tasmania provided the unique opportunity to examine the phenotypic traits of hatchery reared *S. verreauxi* early juveniles from known genetic pools. Although it is possible to produce hatchery reared *J. edwardsii* (Tong et al., 1997 ; Tong et al., 2000 ; Jeffs, 2010), wild emergent juveniles were examined in preference because the long planktonic larval phase which lasts up to two years makes *J.edwardsii* more difficult to culture and less available (Phillips & Sastry, 1980 ; Booth & Phillips, 1994). Investigating both hatchery-produced and wild stock juveniles provides an opportunity to examine how the relationship between physiology, behaviour and feeding traits of individual lobsters and growth may differ between species and environments

The aim of the current chapter is to provide background information on: 1) spiny lobsters, aquaculture practice and issues in their production, 2) the limited available information on abiotic and biotic factors affecting the growth of spiny lobsters, 3) information pertaining to energy metabolism, social behaviour and feeding for fish and crustaceans and 4) the thesis aims and structure.

1.2 Biological characteristics

1.2.1 Systematics and taxonomy

Spiny lobsters, also known as rock lobsters, constitute the family Palinuridae which is in the phylum Arthropoda, class Malacostraca and order Decapoda. Palinuridae is divided into two main evolutionary lineages; Stridentes and Silentes, based on the presence or absence of the stridulating organ which is located at the base of the antennae (Bouwma & Herrnkind, 2009; Staaterman et al., 2010). Stridentes consist of the genera *Panulirus, Justitia, Palinurus, Palinustus, Linuparus, Puerulus* and *Palibythus,* while Silentes consist of the genera *Jasus, Sagmariasus, Projasus* and *Palinurellus* (George, 2006).

Jasus (Parker, 1883) is the most diverse genus of the Palinuridae in the Southern Hemisphere, encompassing five species; *Jasus edwardsii* (Hutton, 1875), *Jasus lalandii* (H. Milne Edwards, 1837), *Jasus paulensis* (Heller, 1862), *Jasus tristani* (Holthuis, 1963) and *Jasus frontalis* (H. Milne Edwards, 1837). However, more recently DNA evidence recommended that *J. paulensis* and *J. tristani* should be synonymized as *J. paulensis* as there

were insufficient differences to differentiate between the species (Groeneveld et al., 2012). All five species were reported to be biologically and morphologically very similar with relatively little genetic divergence among species (Ovenden, et al., 1997).

The genus *Sagmariasus* is represented by a single species, *Sagmariasus verreauxi* (H. Milne Edwards, 1851). Earlier, this species was placed in the genus *Jasus*, but in 2002 was separated to the subgenus *Sagmariasus* because of differences in morphological and behavioural characteristics (Booth et al., 2002; Booth, 2006). This decision has subsequently been validated by molecular genetic data (Palero et al., 2009; Tsang et al., 2009).

1.2.2 Life cycle

The life cycle of *Jasus* and *Sagmariasus* species appears to be relatively long. The eggs hatch as the short-lived (hours) and small (1-2 mm long) naupliosoma stage before moulting into the thin, leaf-like phyllosoma larvae (MacDiarmid, 1985). The phyllosoma develops through 11 distinct morphological stages and 17 moults taking from 8 to 24 months to complete (Booth, 1994). During this phase phyllosoma mainly feed on zooplankton (Phleger et al., 2001). With a limited swimming ability, the planktonic phyllosoma are dispersed long distances offshore before metamorphosing into a completely transparent non-feeding nektonic pueruli which travels from beyond the continental shelf to the coast and settles in shallow water (Booth, 1986; Jeff et al., 2001a). The puerulus resembles the adult form with associated pigmentation occurs 2-4 weeks after metamorphosis and moults into the first instar juvenile with pale colouration that changes to the distinctive colour of the adult after several moults (Edmund, 1995). The first instar juvenile stage mainly feed on molluscs, crustaceans, polychaetes, echinoderms and some algae (MacDiarmid & Booth, 2003; Redd et al., 2008). Juvenile and adult stages live in shallow water, mainly inhabiting rocky reef, sand or mud substrates at a depth of a few metres to about 200 meters (Holthuis, 2002).

a. Eastern rock lobster, *Sagmariasus verreauxi*

Sagmariasus verreauxi can be found in the coastal waters of northern New Zealand and south-east Australia (Kensler, 1967 ; Montgomery et al., 1996 ; Montgomery & Craig, 2005). In New Zealand, *S. verreauxi* is known as the green or packhorse lobster and in Australia it is known as the eastern rock lobster (Kittaka et al., 1997). *S. verreauxi* is an important regional fishery in Australia (New South Wales) and New Zealand (north) (Montgomery & Craig, 2005). It is the largest species of spiny lobster in the world and can grow up to 7 kg and 284 mm in carapace length (Montgomery et al., 2009). Female *S. verreauxi* become sexually mature

at an average of 167 mm carapace length (CL), and can carry up to 1.9 million eggs for several months during the breeding season which only occurs once a year (Kensler, 1967 ; Booth & Phillips, 1994). In winter or early spring, the adults will move inshore to moult, mate and extrude eggs (Booth, 1997).

b. Southern rock lobster, *Jasus edwardsii*

Jasus edwardsii is commonly known as the red rock lobster or southern rock lobster and widely distributed throughout coastal waters of New Zealand (Booth, 2000) and southern Australia (Phillips et al., 2000). This species is an even more important commercially exploited species in Australia and New Zealand (Jeffs et al., 2013). The species can grow up to 235 mm CL for male and 180 CL for female (Holthuis, 1991). Male *J. edwardsii* become sexually mature between 55 and 85 mm CL whereas for females it is between 60 and 120 mm CL (MacDiarmid, 1989; Turner et al., 2002; Annala et al., 1980). Breeding of *J. edwardsii* occurs in winter shortly after the female moults. Spawning of female occurs once a year, in autumn (April to May), and the eggs hatch in spring to summer (September to January) (Booth, 2006).

1.3 Spiny lobsters aquaculture

Spiny lobsters are known as one of the world's most valuable seafood due to a strong and increasing global demand in Asia, Europe and America (Ngoc et al., 2009 ; Hart, 2009 ; Francis et al., 2014 ; Perera & Simon, 2015). The genera *Jasus* and *Sagmariasus* have been the focus of aquaculture research for over 30 years in several counties including Australia, New Zealand and Japan where a significant investment has been devoted to developing hatchery technologies (Kittaka, 1988; Kittaka, 1994; Kittaka & Booth, 1997; Kittaka, et al., 2005; Jeffs, 2010 ; Fitzgibbon & Battaglene, 2012a; Fitzgibbon & Battaglene, 2012b; Jensen, et al., 2013a; Jensen, et al., 2013b; Jeffs et al., 2013; Fitzgibbon, et al., 2017). The development of effective closed-cycle spiny lobster culture has been hampered by the long and complicated larval culture (Kittaka & Booth, 2008 ; Ngoc et al., 2009 ; Jeffs, 2010). A relatively small number of spiny lobsters from the genera *Jasus* and *Sagmariasus* have been reared from egg to juvenile in laboratories (Kittaka, 1988 ; Illingworth et al., 1997 ; Kittaka, 1997 ; Kittaka et al., 1997 ; Tong et al., 2000 ; Kittaka et al., 2005 ; Kittaka & Booth, 2008; Fitzgibbon & Battaglene, 2012a; Fitzgibbon & Battaglene, 2012b; Jensen, et al., 2013a; Jensen, et al., 2013b; Fitzgibbon, et al., 2017). Presently, *S. verreauxi* and *J. edwardsii* are both successfully cultured from egg to adult at a research scale in Australia (G. Smith, personal communication, Institute of Marine and Antartic Studies, University of Tasmania). However, further research and development on

the larval culture of these spiny lobsters are required before successful commercial-scale production.

The sustainable development of spiny lobster aquaculture sector worldwide depends on having a reliable supply of seed. Currently, the entire global lobster aquaculture practice of spiny lobsters is based on the on-growing of wild-caught stock (pueruli and early juveniles) from fisheries which are now either at their maximum sustainable yield or overexploited and in decline (Phillips, 2000 ; Phillips, 2005 ; Jeffs et al., 2013 ; Francis et al., 2014). In countries such as Vietnam and Indonesia, juvenile spiny lobsters are collected from the wild and stocked into floating sea cages then fed with trash fish for several months until they reach marketable size (Hung & Tuan, 2008 ; Priyambodo, 2008 ; Long & Hoc, 2009 ; Ngoc et al., 2009). Sea cage production is the preferred method of culture, especially in South East Asian countries, due to its low capital cost and its effectiveness as a juvenile lobster grow-out system. In Australia, this system is not as practical due to a limitation in infrastructure and other factors such as biological (predators), physical (tidal, cyclones), geographical, cultural and environmental impact (Kenway et al., 2009). Hence, intensive land-based culture is a more preferred method in developed countries such as Australia, as this allows better control of culture parameters and environmental impact. However, further research is required to improve growth and production efficiencies to make it economically feasible.

1.3.1 Growth of spiny lobsters

In arthropods, growth is defined as the accumulation of new tissue which involves a continuous process that only becomes apparent when the animal moults by shedding its old rigid exoskeleton and expand its size during the process called ecdysis. The growth rate of an individual lobster is the outcome of two factors; (1) the time from one ecdysis to the other or known as the intermoult period, and the size increase at ecdysis or described as moult increment. Moult increment is usually measured as carapace length or as body weight. Moult frequency decreased as the individual lobster age increases (Phillips & Sastry., 1980). According to Travis (1954), smaller lobsters are reported have greater percentage of growth by weight. However, as the lobster moult, larger lobsters gain more actual weight in comparison to smaller lobsters.

Studies have reported regional and temporal variability in growth, development and the size at sexual maturity in lobsters which have mainly been attributed to environmental variation (Wahle & Fogarty, 2006). However, even within offspring from the same maternal source and reared under identical conditions, significant individual variability in growth can be observed

(Aiken & Waddy, 1988). Waddy et al. (1995) suggested that small differences in initial size at hatch may be an important factor in determining individual growth trajectories when lobsters are reared communally. Other factors that have been reported to play a role in individual lobster growth include temperature, light and photoperiod, nutrition and food availability, stocking density, space and shelter, and behavioural and social conditions (Tong et al., 2000 ; Thomas et al., 2000 ; Crear et al., 2000 ; Thomas et al., 2003 ; Bryars & Geddes, 2005 ; Simon & James, 2007 ; Perera et al., 2007 ; Fitzgibbon & Battaglene, 2012b ; Carter et al., 2014 ; Fitzgibbon et al., 2017). Prolonged inter-moult duration, reduced growth increment, and even shrinkage can result from stressful environmental conditions (Cockcroft & Goosen, 1995 ; Irvin & Williams, 2008), which may have important implications for population dynamics and management.

The most common practice in most spiny lobster culture is to hold and rear the lobsters communally. Previous research on *P. ornatus* has demonstrated that lobsters in communal culture grow significantly faster than those reared individually, however, the survival rate of individually cultured lobsters was higher, probably due in part to the lack of cannibalism (Irvin & Williams, 2008 ; Ratunil Jr, 2017). One of the major issues in the juvenile lobster grow-out system is the increase in variation of a population size distribution with time due to individual differences in growth rates leading to growth disparity and growth depensation and, impacting on biomass production and uniformity of production in aquaculture (Irvin & Williams, 2008 ; Vijayakumaran et al., 2010 ; Carter et al., 2014 ; Ratunil Jr, 2017). According to Thomas et al. (2003), growth disparity in spiny lobsters is linked to the agonistic behaviour of dominant individuals, whereby they control and consume a disproportionate share of food resources, benefiting their growth performance. Further research is required to understand the basis for individual differences in behavioural traits and how it can influence growth disparity and depensation to improve lobster welfare and increase the profit of production.

1.4 Energy metabolism

Aerobic energy metabolism can be categorised and often measured at various levels of activity (Cockcroft & Wooldridge, 1985). These levels of energy metabolism are also known as metabolic rates which includes the baseline or resting metabolic rate which is termed the basal metabolic rate (BMR) in homeotherms and standard metabolic rate (SMR) in ectotherms. The other rates are the routine metabolic rate (RMR), active metabolic rate (AMR) and aerobic scope (AS) (Metcalfe et al., 1995 ; Biro & Stamps, 2010 ; Auer et al., 2015a ; Auer et al., 2016).

The SMR is an estimation of a minimum metabolic rate where the individual animal is in a post-absorptive and inactive state (Chabot et al., 2016). It is the most useful measurement in intra and inter-specific comparisons because it allows a standard reference of an unstressed animal for species comparisons (Radull et al., 2002). The SMR can be measured when an animal is inactive without metabolic costs associated with digestion, stress and previous anaerobic activity (McNab, 1988 ; Hulbert & Else, 2000 ; Frappell & Butler, 2004). During measurement of SMR, the energy used is for processes such as the maintenance of the mitochondrial H+ gradient, protein turnover, repair or turnover of cellular structures, active transport of solutes, blood circulation and ventilation (Rolfe & Brown, 1997 ; Hulbert & Else, 2000).

Routine metabolic rates (RMR) includes by definition costs for spontaneous activities. The RMR is measured when the animal is still inactive but not necessarily at the lowest rate (Biro & Stamps, 2010). Active metabolic rate (AMR) is the maximum rate of aerobic metabolism and is a measure of the maximal level of oxygen consumption (Bennett, 1978). The most accurate method to measure AMR in crustacean is using chase protocols where the animal is exercised to near exhaustion (Booth & McMahon, 1992 ; Jimenez et al., 2008 ; Fitzgibbon et al., 2014b). Animals (including lobsters) typically display an increase in metabolic rate which is referred as excess post-exercise oxygen consumption (EPOC) which is above SMR after the chase exercise (Lee et al., 2003 ; Fitzgibbon et al., 2014b). The maximum metabolic rate under this condition is equivalent to the aerobic capacity (Steffensen, 2002 ; Fitzgibbon et al., 2014b). Aerobic scope (AS) is the capacity to perform oxygen-consuming processes above the minimum metabolic requirements and the ability to compensate for physiological challenges (Djawdan et al., 1997; Bochdansky et al., 2005; Killen et al., 2007).

Metabolic rates (MR) of individuals are not constant and there are many factors that have been shown to affect MR of aquatic animals including temperature, body size, life history, lifestyle (benthic vs pelagic) and food availability (Jobling, 1981; Thomas, et al., 2000; Dowd et al., 2006; Ohlberger et al., 2007; Perera et al., 2007; Seppänen, et a., 2010, Killen et al., 2010; Vijayan et al., 2010; Auer et al., 2016). In spiny lobsters, various factors have also been studied and confirmed to affect lobster MR including temperature, animal size, feeding activity, stocking density and moulting cycle (Buesa, 1979 ; Crear & Forteath, 2000 ; Fitzgibbon & Battaglene, 2012b ; Jensen et al., 2013a ; Jensen et al., 2013b ; Fitzgibbon et al., 2017). In *J. edwardsii*, SMR typically increases exponentially with temperature where the oxygen consumption increases approximately five times from 0.01 mg O_2 /g/h at 5^{o}C to 0.05 mg O_2 /g/h at 21^{o}C (Crear & Forteath, 2000). Metabolic rates of an animal are related to body mass

by an allometric relationship (Schmidt-Nielsen, 1972). According to Rosenfeld et al. (2015), the mass-specific metabolic rate declines through ontogeny as mass approaches adult size. In juvenile *S. verreauxi*, the SMR and AMR are reported to be strongly related to body mass with an allometric scaling exponent of 0.91 and 0.81, respectively (Jensen et al., 2013a).

Metabolic phenotypes are reported to be a key physiological trait which can determine the performance of an organism (Metcalfe et al., 1995; Brown et al., 2003 ; Biro & Stamps, 2010 ; Killen et al., 2014; Killen et al., 2016b; Metcalfe et al., 2016a; Killen et al., 2017). Individuals within a species can also differ widely in their rate of energy metabolism (Burton et al., 2011 ; Auer et al., 2015a ; Metcalfe et al., 2016a). For example, research by Burton et al. (2011) and Metcalfe et al. (2016a) reported that metabolic phenotypes of fish could vary up to threefold among individuals within the same cohort even after correcting for the effect of size, age and sex. Metabolic phenotypes have also been linked with mechanisms that can influence the fitness of an organism such as growth, reproduction, survival, dominance and lifespan (Metcalfe et al., 1995 ; McCarthy, 2000 ; Perera et al., 2007 ; Moltschaniwskyj & Carter, 2010 ; Burton et al., 2011 ; Metcalfe et al., 2016). Individual metabolic rate may also influence locomotor ability (Reidy et al., 2000), dominance and aggression (Brown et al., 2003 ; Biro & Stamps, 2010) which may then affect individual food consumption and growth (Millidine et al., 2009 ; Auer et al., 2015a ; Auer et al., 2015c). However, the relationship between metabolic phenotypes and fitness can differ depending on environmental conditions (McKechnie, 2008). An environment with an abundance of food, such as in captivity can be a benefit for individuals with a high SMR (McCarthy et al., 1994 ; Millidine et al., 2009). Conversely, a low SMR can be beneficial under unfavourable conditions, i.e. food scarcity, (Álvarez & Nicieza, 2005).

Earlier research on teleosts has demonstrated that fish with higher metabolic phenotypes can be more dominant, aggressive, have greater feeding capacity and grow faster resulting in a size advantage, higher rank in a social hierarchy and promoting growth disparity within populations when reared in captivity (McCarthy, 1994 ; Metcalfe et al., 1995 ; Yamamoto et al., 1998 ; Millidine et al., 2009 ; Auer et al., 2015a ; Auer et al., 2015b ; Auer et al., 2015c ; Auer et al., 2016b). Likewise, research on crustaceans such as giant freshwater prawn, *Macrobrachium rosenbergii,* has also revealed that metabolic phenotypes can be an important factor influencing social interactions between individuals within populations, such as fighting, which may affect their growth and survival in rearing (Smith & Taylor, 1993 ; Thorpe et al., 1995 ; Taylor et al., 2002 ; Brown et al., 2003). Though extensive studies have been conducted to investigate the effect of metabolic phenotypes on teleost and crustaceans'

performances (behaviour, growth and feeding) there was to my knowledge no information available on how spiny lobsters metabolic phenotypes may influence individual lobster performances. Further research was needed to understand the basis for individual variation in spiny lobsters metabolic phenotypes and how it could possibly influence individual growth performance and growth disparity when reared in captivity.

1.4.1 Measurement of metabolic rates

In crustaceans, static (closed) respirometer systems have been used to measure respiration rates, where the oxygen level is measured only at the start and end of an experiment (Steffensen, 1989 ; Anger, 1996 ; Bermudes & Ritar, 2008 ; Fitzgibbon & Battaglene, 2012b). Static respirometry provides an average measurement of MR over long periods by comparing the initial oxygen concentration of the water with the final oxygen concentration. Thus, by using this method, it is impossible to segregate periods of rest from periods of high activity. Therefore, measurements made in static respirometer systems are at an intermediate routine metabolic rate (RMR) (Schmidt-Nielsen, 1997). Static respirometry may be appropriate to use to estimate SMR for inactive marine species under conditions of zero swimming activity (when activity levels are monitored) (Killen et al., 2007).

However with lobsters, there may be substantial variation in RMR due to changes in behaviour between inactive to short periods of activity (Perera et al., 2005). The RMR can differ depending on an animal's state of activity, and this can lead to considerable experimental error (Anger, 1996).

To determine the SMR for free-swimming aquatic animals like fish, respirometer systems such as flow-through respirometers or swim tunnel respirometer can be used. By using flow-through respirometer or swim tunnel respirometer, the extrapolation of metabolism at various levels from forced activity to zero activity can be used to determine SMR (Buesa, 1979 ; Crear et al., 2000 ; Ohlberger et al., 2007). Intermittent flow-through respirometry is an alternative system which helps to eliminate problems related with periods of activity and permits repeated measurement of MR during short time intervals over extended periods which allows more accurate estimation of SMR (Forstner, 1983 ; Steffensen, 1989 ; Steffensen, 2002 ; Meskendahl, 2013). This system allows the animal to have adequate acclimation, avoid the accumulation of excretory products as well as prevents large changes in oxygen concentrations (Steffensen, 1989). Intermittent flow-through respirometry has been used to examine the metabolism of several spiny lobsters such as *S. verreauxi* (Fitzgibbon, 2010 ; Jensen et al., 2013c), *J. edwardsii* (Crear & Forteath, 2000) and east coast rock lobster *Panulirus homarus*

*rubellus (*Kemp et al., 2009). An automated intermittent flow-through respirometry system described in the study of *S. verreauxi* pueruli was effective in making accurate repeat measurements of respiration over extended periods facilitating an advanced examination of pueruli metabolism (Fitzgibbon, 2010 ; Fitzgibbon & Battaglene, 2012a ; Fitzgibbon et al., 2014a ; Fitzgibbon et al., 2017).

1.5 Social behaviour

Social interaction plays a significant role in the life history of lobsters as it is important for the survivorship, growth, reproduction, movement during different season and life history stages (Atema & Cobb, 1980 ; Herrnkind, 1980 ; Lawton & Lavalli, 1995). Social behaviour refers to any form of behaviour that involves interaction and communication between two or more individuals of the same species (Atema & Cobb, 1980).

Agonistic behaviour is a part of social behaviour which involves fighting between two or more individuals. Animals often show agonistic behaviour when there are limited resources such as food, shelter and mates (Atema & Cobb, 1980 ; Davis & Olla, 1987 ; Ryer & Olla, 1996). Some animals show agonistic behaviour as a test of strength or threat display to make them look large, physically fit and dominant. Agonistic behaviours vary among species and consist of three types of behaviours; threat, aggression and submission. Agonistic interaction can range from a fight to the death depending on the availability and importance of a resource (Cobb & Phillips, 1980). Aggression refers to offensive behaviour whereas aggressiveness, and aggressive motivation are similar terms used to explain the state of the animal and the probability of it winning an agonistic encounter (Cushing & Reese, 1998). Dominance is the status of an animal describing the likelihood of it succeeding in encounters with conspecifics, and can be characterized by the behavioural differences between individuals. Reproductive cycle and community structures are two essential parts of social behaviour (Cobb & Phillips, 1980).

Spiny lobsters tend to congregate in conspecific groups in suitable shelters. However, spiny lobsters can be quite aggressive and will show agonistic behaviours when they need to find and retain shelter, feeding and mating (Fielder, 1965a ; Fielder, 1965b; Berrill, 1976 ; Cobb, 1981 ; Lozano-Alvarez & Briones-Fourzán, 2001 ; Segura-García et al., 2004 ; Weiss et al., 2006 ; Briones-Fourzán et al., 2008 ; Briones-Fourzán et al., 2014). Agonistic behaviours showed by *J. edwardsii* include aggression, avoidance, attack and some other behaviours such as approach or displacement (Carter et al., 2014). Analysis of the aggressive behaviour of spiny lobsters has been studied in several spiny lobsters including *J. edwardsii*,

J. lalandii, P. cygnus, P. guttatus, P. longipes and *P.argus* (Fielder, 1965a; Fielder, 1965b; Berrill, 1976; Cobb, 1981; Thomas et al., 2003; Segura-García et al., 2004; Moyle et al., 2009; Carter et al., 2014; Briones-Fourzán et al., 2014; Lipcius & Herrnkind, 1982).

In spiny lobsters, an aggressive encounter is initiated by the approach of one animal toward another (Cobb & Phillips, 1980 ; Butler et al., 1997 ; Barshaw et al., 2003). The aggressive state can be observed from the postures of the two animals including the position of the abdomen, pereiopods, urupods and pleopods. Lobsters typically display dominance by standing high on their legs with the abdomen and uropods extended, the telson and uropods held in the horizontal plane, and pleopods extended downwards. Defeated lobsters often show a submissive posture by standing low on their legs, with the tail tucked tightly under the body and uropods and pleopods folded. A dominant spiny lobster will stand high on its legs with abdomen, pleopods, and uropods extended and the tail fan held slightly below the horizontal plane when it is approaching a conspecific. The lobster being approached may either show a similar posture or will avoid the approaching animal by walking away low on its legs with tailed curled. During the approach, one or both lobsters may use the antennae or antennules to contact the other lobster. During an aggressive approach, the most common postures are chase, clasp and flee. Termination of an encounter is marked when one of the lobsters retreats by walking away forward or backward, or tail flips in retreat (Atema & Cobb, 1980; Cobb, 1981; Segura-García et al., 2004; Carter et al., 2014).

1.5.1 Dominance and hierarchy

An outcome of encounters between animals is influenced by many factors such as size, sex, moult stage, recent agonistic experience and energy metabolism (Karavanich & Atema, 1998). According to Fielder (1965b), larger males of *J. lalandii* are able to dominate and retain shelter over smaller lobsters. In *H. americanus* and *J. edwardsii*, individual size was found to be the primary factor influencing the aggressive rank with males conferring an advantage (Roth, 1972 ; Cobb et al., 1982 ; Thomas et al., 2003). Larger individuals are often found to become more dominant and aggressive, able to consume more food and grow faster than the smaller (subordinate) individuals (Thomas et al., 2003). In *J. edwardsii*, the larger individuals are regularly able to maintain their dominant status when feeding opportunities are restricted. However, as feed availability increased, fewer of the initially largest size-ranked lobsters maintained their status (Thomas et al., 2003).

Other factors, such as recent agonistic experience also play a role in the determination of dominance. Karavanich and Atema (1998) investigated how *H. americanus* maintain their

stable dominance relationship. The results suggest that *H. americanus* are capable of individual recognition where subordinates will immediately back away from familiar dominants and avoid the second fight when the lobsters are paired (dominant and subordinates). In contrast, studies with crayfish, *Procambarus clarkii* shows that the formation of linear dominance hierarchies does not involve learned individual recognition (Copp, 1986).

Moulting status has also been found to be one of the factors influencing the aggressive rank with moulting activity typically suppressing aggressiveness of dominant individuals. The aggressive rank of *P. cygnus* individuals that have just moulted, or are about to moult, was found to markedly drop and remain at the lowest rank for 2 or 3 days after moulting until the exoskeleton hardened (Cobb & Phillips, 1980). Juveniles of *M. rosenbergii* lost more fights before the moult, however, were able to retain rank after moulting (Bovbjerg, 1953 ; Brown et al., 2003).

Dominant and subordinate animals have different behaviours. Studies on a group of *P. cygnus* showed that the lowest ranked individual displayed submissive behaviour by not making any attempts to enter the shelter and avoiding all other approaching animals. Meanwhile, the dominant lobsters had access to all shelters and moved around the entire aquarium (Cobb & Phillips, 1980). The higher ranked spiny lobsters also have the advantage in gaining food by preventing the subordinate's food intake by behaviourally inhibiting the subordinate's feeding behaviour (Koebele, 1985).

In fish and crustaceans such as Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and giant freshwater prawn *M. rosenbergii*, energy metabolism is one of the factors which contribute to the dominant-subordinate relationship (Brown, 1946 ; Metcalfe et al., 1995 ; Brown et al., 2003). But no comparative studies on the effects of energy metabolism on the strength of dominance order in spiny lobsters have been conducted. It has been demonstrated in *S. salar* and *M. rosenbergii* that energy metabolism was significantly correlated with dominance status with animals with higher SMR, being more dominant regardless of individual sex, body mass and date of first feeding (Metcalfe et al., 1995 ; Yamamoto et al., 1998). More dominant individuals grow faster, and their resulting size advantage tends to strengthen the social hierarchy.

1.6 Feeding

1.6.1 Feeding preference

Feeding plays an important role in determining the success of spiny lobster culture. Knowing the food preference of spiny lobsters in the wild is useful information when providing the best diet and developing an artificial feed, and to improve their feeding performance such as feeding intake and feeding capacity which indirectly may improve their growth performance. In the wild, the natural diet of spiny lobsters predominantly consists of molluscs, crustaceans, polychaetes, echinoderms and some algae (Edmunds, 1995; Saunders et al., 2012; Jeff et al., 2013). In most spiny lobster culture practice particularly in South East Asia, fresh fish by-catch has been used as the main natural food source (Williams, 2009). However, previous studies have demonstrated that mussels are an excellent natural food source for spiny lobster (James & Tong, 1997; Williams et al., 2005 ; Simon & James, 2007 ; Williams, 2009 ; Francis et al., 2014 ; Perera et al., 2005 ; Perera & Simon, 2015). In the wild the natural diet of spiny lobsters consists of wide variety of food types, and earlier research has shown that spiny lobsters are "picky eaters" being selective towards their preferred food (Eurich et al., 2014 ; Williams, 2009 ; Williams et al., 2005). Previous studies have also proven that spiny lobsters were more attracted to fresh mussels over artificial diets (Crear et al., 2000 ; Tolomei et al., 2003 ; Dubber et al., 2004 ; Williams et al., 2005 ; Simon & James, 2007), and improved their food consumption and growth performance when fed on mussels compared to formulated feeds (Tsvetnenko et al., 1999 ; Williams et al., 2005 ; Dubber et al., 2004 ; Simon & James, 2007). Fresh mussels provide better performance due to their nutritional composition as well as other factors such as digestibility and nutrient assimilation (Williams, 2009).

1.6.2 Feed intake

Studies on feed intake of spiny lobsters have examined the effect of abiotic and biotic factors and the correlation with lobster growth performance (Lipcius & Herrnkind, 1982 ; Lellis & Russell, 1990 ; Simon & Jeffs, 2011 ; Simon, 2009 ; Fitzgibbon & Battaglene, 2012b ; Fitzgibbon et al., 2017). While research has been done extensively to investigate factors that may affect spiny lobsters feed intake, there is still no information available about the effect of metabolic phenotype on spiny lobsters feed intake. Previous studies on fish have shown that maximum feed intake is correlated with individual metabolic phenotype, where individuals with higher AS were able to ingest more food per day relative to individuals with a lower AS (Auer et al., 2015a). Higher SMR and AS individuals may also be able to take advantage of high food abundance due to their ability to digest a meal more quickly, which implies they may

be able to consume more food per day (Auer et al., 2015a ; Auer et al., 2015b ; Auer et al., 2015c; Killen et al., 2016a). Earlier studies demonstrated that fish with higher metabolic rate tend to grow faster in high food environments (Rosenfeld et al., 2015 ; Metcalfe et al., 2016a) because of their high cost of maintenance and higher assimilation efficiency (converting ingested food to energy for growth and reproduction) requiring greater amounts of food consumption to uphold their large "metabolic machinery" (Van Dijk et al., 2002 ; Hou et al., 2008 ; Millidine et al., 2009 ; Biro & Stamps, 2010 ; Auer et al., 2015b ; McKenzie et al., 2015 ; Allen et al., 2016 ; Killen et al., 2016a).

1.7 **Thesis aim and structure**

The successful production of spiny lobsters in captivity has been hampered by the large inter-individual variation in growth rate leading to growth disparity and ultimately reduced lobster biomass. One explanation for the growth disparity in spiny lobsters is the agonistic behaviour of dominant individuals, whereby they control and consume a disproportionate share of food resources, benefiting their own growth performance. Nevertheless, the mechanisms of how behavioural and feeding traits of individual lobsters can affect growth disparity in culture are poorly understood. Previous research of a range of marine organisms has hypothesised that behaviour and growth can be influenced by the variability in individual metabolic physiology. However, the correlation between metabolic physiology and individual growth performance has not been previously investigated in any spiny lobster species. Thus, the ultimate aim of this thesis is to understand the influence of intraspecific diversity in physiological traits, behaviour and feeding on growth performance of individual spiny lobsters in captivity. These are important considerations for the development of optimal rearing conditions and management strategies for spiny lobsters aquaculture. This is the first study to focus on the influence of individual variation in physiology, behaviour and feeding on the growth performance of two commercial temperate spiny lobsters species, *Sagmariasus verreauxi* and *Jasus edwardsii* juveniles in captivity. The research has been approached using paired tests of individually and communally held lobsters (both wild and cultured) and at various stages of development and measured their individual metabolic phenotype, food consumption, social status and growth. This objective has been addressed in four research chapters that have been structured as a journal manuscript for submission to scientific journals. Consequently, some of the chapter content may be repeated, particularly in the introduction and materials and methods sections.

Chapter 2 to investigate the influence of metabolic phenotype and social behaviour on growth performance of early juvenile *S. verreauxi* that were reared either individually or communally.

Chapter 3 to examine the effect of individual variation of metabolic phenotype, body size, sex, feeding contest experience and rearing history on social status of early juvenile *S. verreauxi* using a pair-feeding contest behavioural studies.

Chapter 4 to examine the influence of intraspecific body size variation, metabolic phenotype and social behaviour on growth performance of emergent juvenile *J. edwardsii* that were reared either individually or communally.

Chapter 5 to investigate the relationships of metabolic phenotype on individual feed intake and growth performance of the emergent juvenile of *J. edwardsii*, and identify the food preference amongst current best diets of the emergent juveniles using a multiple-choice feeding experiment and linked with individual growth performance.

Chapter 6- This uniting chapter highlights the key findings and provides a general discussion that synthesizes major findings from all research chapters (Chapter 2- 5) while providing a brief discussion on the limitation of the research approach, and future research directions.

CHAPTER 2

IS INDIVIDUAL VARIATION IN METABOLIC RATE RELATED TO GROWTH OF SPINY LOBSTER IN CULTURE AND WHAT IS THE INFLUENCE OF SOCIAL INTERACTION?

2.1 ABSTRACT

Slow growth, growth disparity and growth depensation have been reported as major drawbacks to the successful production of spiny lobsters in captivity. This is thought to be associated with agonistic behaviour of dominant individuals controlling a disproportional share of food resources compared to the subordinates. Previous research with variety of aquatic ectotherms suggest that variation in individual metabolic rate (i.e., metabolic phenotype) can be a factor that determines the behaviour and growth of individuals, however, the relationship has not been previously examined in any spiny lobster species. This study examined the relationship between individual variation of metabolic phenotypes (standard, routine and active metabolic rates and aerobic scope), and growth performance of juvenile spiny lobster, *Sagmariasus verreauxi* (5.99±0.46g) that were reared either individually (n=17) or as a group of 20 communally for 90 days. Growth performance, survival and feed intake were significantly higher in communal rearing demonstrating that social interaction is important for promoting growth of lobsters. There was a positive relationship between standard metabolic rate, routine metabolic rate and growth in individually reared lobsters indicating a direct link between metabolic phenotype and growth of lobsters in the absence of social interaction. The effect of social interaction in communal rearing outweighed the direct link between metabolic rate and lobster growth. The results demonstrate that growth performance of spiny lobsters is linked with individual variation in metabolic status with social behaviour playing an important role in determining the growth of individuals.

Keywords: Social behaviour, metabolic rate, growth, individual variation

2.2 INTRODUCTION

Metabolic rate (MR) is considered the aerobic energetic cost of living which is required for an organism to perform, process and function in order to support life (Hulbert & Else, 2000). At the very minimum, an individual must expend energy to sustain life and maintenance of tissue (Secor, 2009). This baseline energy expenditure is known as standard metabolic rate (SMR) in aquatic ectotherms (Auer et al., 2015a). The average rate of metabolism when the animal is undergoing minimum motor activity is known as routine metabolic rate (RMR) (Metcalfe, 2015) whereas the upper boundary for aerobic energy metabolism is known as active metabolic rate (AMR) (Burton et al., 2011 ; Auer et al., 2015a). By subtracting the minimal from the maximal MR, the total amount of aerobic energy available to the animal can be measured. This measurement is known as aerobic scope (AS) (Auer et al., 2015a ; Metcalfe et al., 2016a ; Killen et al., 2007 ; Norin & Malte, 2011 ; Clark et al., 2013).

Metabolic rate is a key physiological trait which can determine the performance of an organism (Carter & Brafield, 1991 ; Brown et al., 2003 ; Biro & Stamps, 2010). Most active organisms such as pelagic fish tend to have a higher MR compared to inactive species. (White & Seymour, 2004). For some fish and crustaceans, MR can be heritable (Briffa et al., 2008 ; Nilsson et al., 2009 ; Wone et al., 2009) and repeatable throughout time (McCarthy, 2000 ; Labocha et al., 2004 ; Nespolo & Franco, 2007 ; Maciak & Konarzewski, 2010 ; Norin & Malte, 2011). Individuals within a species can also differ widely in their rate of energy metabolism, known as metabolic phenotype (Burton et al., 2011 ; Auer et al., 2015a ; Metcalfe, 2015). Burton et al. (2011) and Metcalfe (2016a) reported that metabolic phenotype of can be vary up to threefold among individuals within the same cohort even after correcting for the effect of size, age and sex.

Metabolic phenotypes have been linked with mechanisms that can influence the fitness of an organism such as growth, reproduction, survival, dominance and life span (Metcalfe et al., 1995 ; McCarthy, 2000 ; Perera et al., 2007 ; Moltschaniwskyj & Carter, 2010 ; Burton et al., 2011 ; Metcalfe et al., 2016b). Individual metabolic rate may also influence locomotor ability (Reidy et al., 2000), dominance and aggression (Brown et al., 2003 ; Biro & Stamps, 2010) which may then affect individual food consumption and growth (Millidine et al., 2009 ; Auer et al., 2015a ; Auer et al., 2015b). However, the relationship between metabolic phenotype and fitness can differ depending on environmental conditions (McKechnie, 2008). An environment with abundant of food, such as in captivity can be a benefit for individuals with a high SMR (McCarthy, 1994 ; Millidine et al., 2009). Conversely, a low SMR can be beneficial under unfavourable conditions, i.e. food scarcity, (Álvarez & Nicieza, 2005).

Previous studies of teleosts in captivity have shown that individuals with higher metabolic rate can be more dominant, aggressive and grow faster resulting in a size advantage that ensures a higher rank in a social hierarchy (McCarthy, 1994 ; Metcalfe et al., 1995 ; Yamamoto et al., 1998 ; Millidine et al., 2009). Similarly, studies on crustaceans such as giant freshwater prawn, *Macrobrachium rosenbergii*, have also shown that rates of both aerobic and anaerobic metabolism can be important in social interactions, such as fighting, which may affect their growth and survival in rearing (Smith & Taylor, 1993 ; Thorpe et al., 1995 ; Taylor et al., 2002 ; Brown et al., 2003).

Spiny lobsters are known to be aggressive and highly gregarious animals with complex social behaviours which they use for the development of social hierarchies (Shabani et al., 2009), shelter selection (Segura-García et al., 2004; Briones-Fourzán et al., 2008), predator avoidance (Briones-Fourzán et al., 2014) and even behavioural immunity (Anderson and Behringer, 2013; Butler et al., 2015; Candia-Zulbarán et al., 2015) in the wild. In the rearing of spiny lobsters, slow growth and great growth disparity and depensation have been reported as the major drawbacks to the successful production in captivity (Irvin & Williams, 2008 ; Carter et al., 2014). Thomas et al. (2003) reported that the growth depensation in spiny lobster rearing is often associated with hierarchical social structure and agonistic behaviour where the dominant individuals control a disproportionate share of food resources compared to the subordinates. Due to differences in food intake, individuals grow at different rates which can cause an increase in variance of a size distribution with time. The relationship between metabolic phenotype, dominance behaviour and growth of spiny lobsters in rearing has not been previously examined. The present study aimed to examine the influence of metabolic phenotype and growth performance of juvenile eastern rock lobster, *Sagmariasus verreauxi* in captivity.

Sagmariasus verreauxi is an emerging aquaculture species resulting from breakthroughs in hatchery production (Fitzgibbon & Battaglene, 2012a ; Fitzgibbon & Battaglene, 2012b). In the present study, hatchery produced juvenile lobsters were reared either individually or communally in order to determine the potential influence of social behaviour on the relationship between metabolic phenotype and growth performance. This present study hypothesized that dominance hierarchies and growth disparity are related to metabolic phenotype, with individuals with higher metabolic rates being more dominant and growing faster in communal rearing. An improved understanding of the relationship between metabolic phenotype and growth may be important for determining causes of growth disparity and depensation in rearing which are a significant impediment for uniformity of production.

Furthermore, information on individual performance in rearing may allow improved selection of broodstock in a selective breeding program to optimize growth and uniformity of production.

2.3 MATERIALS AND METHODS

2.3.1 Experimental animals

Early stage juvenile *S. verreauxi* were reared from eggs at the Institute for Marine and Antarctic Studies, Hobart, Australia. Larvae were hatched on the 11th of January 2013 from captive wild -caught females and hatchery reared to the final instar phyllosoma as described by Fitzgibbon and Battaglene (2012b). After metamorphosis, pueruli were communally reared in a 100-l cylindrical vessel which received flow-through filtered sea water at three exchanges per hour and maintained at 21^{o}C with a light regime of 16 h light and 8 h dark. No feed was provided to the lecithotrophic puerulus stage. First instar juveniles were then transferred into a 100-l rectangular rearing vessel which received flow-through filtered seawater at three exchanges and fed once per day with split fresh blue mussels (*Mytilus galloprovincialis*) to excess. All uneaten feed was removed from the rearing system before subsequent feeding and tanks were cleaned weekly.

Before commencement of the experiment, sixty juvenile lobsters (300 days post hatching) were randomly removed from mass rearing and placed into two 32-l cylinder tanks (n=30 in each tank) receiving filtered flow through seawater at a temperature of 21±1^{o}C and light regime of 12 h light and 12 h dark for 42 days. During this period, individual oxygen consumption rate ($\dot{M}o_2$) was measured (refers to the $\dot{M}o_2$ method below). During holding, lobsters were fed blue mussels to excess once per day. All uneaten feed was removed from the rearing system immediately before the next feed and faeces were siphoned out from each rearing vessel between 0800 and 0900 h. All lobsters were individually tagged by gluing (Loctite 454 ®) a numbered polymer tag (diameter 5 mm) to their carapace and injecting Visible Implant Elastomer tags (VIS) on the edge of 1st abdominal segments in order to differentiate each juvenile (Woods & James, 2003). After 42 days, 40 lobsters were randomly distributed into the experimental system. At the start of the experiment, all lobsters had a full set of appendages. Lobsters were acclimatised to the experimental system and feeding regime for 1 week prior to the sex determination and initial body weight (BW) and carapace length (CL) measurement. All body weight measurements conducted in this experiment were recorded after drying the juvenile with paper towel.

2.3.2 Individual and communal rearing experiment

The experiment was conducted under two rearing conditions; individual rearing and communal rearing. Both rearing conditions received the same seawater supply at a flow rate of three water exchanges h^{-1}. For individual rearing, 20 lobsters were initially stocked into 20 black cylindrical vessels (13 cm diameter and 12 cm height) with water volume of 1.6 l and floor surface of 132 cm^2, with a single cylindrical shelter made from 3 mm x 3 mm high-density polyethylene oyster mesh (12 cm length x 5 cm diameter) in order to minimise stress. Communal rearing involved 20 lobsters stocked into a single vessel (58 cm diameter and 12 cm height) of similar colour and shape. Stocking density for both treatments were 0.37 lobster m^{-2}. Due to the limited number of experimental animals, the communal rearing could not be replicated. The water volume and surface area was proportionally the same but 20 times greater than that for individual rearing (32 l and 2642 cm^2), with 20 cylindrical shelters provided. The initial coefficient of variation for body weight (CV_w) and carapace length (CV_{CL}) in communal rearing was 46.63% and 15.28%, respectively, whereas in individual rearing it was 44.72% and 13.72%, respectively. All lobsters in both rearing conditions were fed to excess once per day with split fresh blue mussels. In individual rearing, one split mussel per day was fed to each lobster whereas the communal rearing vessel received 20 split mussels per day (i.e. one mussel per lobster per day). One split mussel per individual was established to be excess of satiation for the lobsters examined.

The water quality parameters (pH, dissolved oxygen and temperature) were measured daily and maintained at temperature 20-22^oC, salinity 33-35, pH 8.1 and 80-100% oxygen saturation. Light was provided from fluorescent tubes with a 12:12 photoperiod. Between 08:00 and 09:00 am vessels were checked for moulting or mortality, all moulting and mortality were recorded and removed. Moulted lobsters were identified by identifying juveniles without tag. The new exoskeletons of the moulted lobsters were allowed to harden before weighing BW, measuring CL and re-tagging with numbered polymer tag. All uneaten feed and moults were collected immediately before feeding and faeces were siphoned. The experiment was terminated after 90 days.

2.3.3 Oxygen consumption rate ($\dot{M}O_2$)

Before commencement of the rearing experiment the oxygen consumption rate ($\dot{M}O_2$) of all lobsters was measured using automated intermittent flow-through respirometry similar to that described by Fitzgibbon et al. (2014a). The respiratory system comprised of four 50 ml polypropylene respiratory chambers (50 ml conical bottom centrifuge tubes) with internal

diameter of 30 mm and a length of 115 mm submerged in a water bath (24 cm height X 24 cm length X 25 cm width) that received seawater continuously at a 17 l h^{-1} from a 500 l insulated sump which was heated to the treatment temperature (21±0.2^{o}C) by an emersion heater. Two twin channel mini peristaltic pumps (Harvard MP II mini-peristaltic pump) were used to continuously circulate water at a rate of 10 ml min^{-1} through the chambers and past an oxygen sensor. Dissolved oxygen was recorded and logged every 20 s by a fibre optic oxygen microsensor meter (OXY- 4 mini, www.preSense.de) connected to a computer. Another two twin channel peristaltic pumps were used to introduce new water from the external water bath at the rate of 14 ml min^{-1}. A digital recycler timer (Sentinel DRT-1) was connected to the pumps and was programmed to turn on in 10 min and 5 min off cycles which allowed a $\dot{M}o_2$ measurement every 15 min. Air through an aquarium air stone maintained the dissolved oxygen concentration in the external water bath at 100% saturation. A black corflute screen was used to enclose the water bath housing in order to exclude light and external stimuli. Dissolved oxygen within the respiratory chambers never fell below 85% throughout the $\dot{M}o_2$ measurements. The respiratory system was sterilized with a 1 mg l^{-1} solution of sodium hypochlorite, rinsed with fresh water, and air dried following each experiment.

The oxygen consumption rate evaluations and metabolic states of all lobsters were evaluated (see Fitzgibbon et al. 2014a). In the 42 days leading up to the rearing experiment, the moulting of all lobsters was monitored and $\dot{M}o_2$ of individuals were measured during the intermoult phase which was defined from day 7 to 15 post moult. Before $\dot{M}o_2$ measurement the lobsters were starved for 24 hours to clear the digestive tract of food and faeces, and to eliminate the thermic effect of food (total energy expenditure above the SMR due to the cost of processing food for use and storage) or also known as specific dynamic action (SDA). In the late evening after 24 h of starvation, individuals were placed into the respirometer chambers and $\dot{M}o_2$ logged overnight for 16 h for approximately 64 $\dot{M}o_2$ measurements. The mean of the lowest five recording of the $\dot{M}o^2$ was defined as standard metabolic rate (SMR) and the average of all 64 recordings was defined as routine metabolic rate (RMR). To stimulate active metabolic rate (AMR), lobsters were removed from the respirometer and made to swim by encouraging the lobster by hand to swim inside a 40 cm height x 60 cm length x 30 cm width vessel until it became exhausted and non-responsive to stimuli (approximately 10 min). Lobsters were then placed back into the chamber and $\dot{M}o_2$ recorded for 2 h. The exhaustion protocol was maintained to keep in time with the open cycle of respirometer system to allow immediate $\dot{M}o_2$ measurements. The highest 10% recordings of the oxygen consumption rate measured after the exhaustive exercise was defined as active metabolic rate (AMR). Aerobic scope (AS) was

determined by subtracting the SMR from the AMR. All lobsters were removed from the chamber immediately after respiratory measurement was completed and body weight was recorded. Oxygen demand of the respirometer system was then recorded for another 1 to 2 h as a measurement of background respiration. Lobster $\dot{M}o_2$ were determined using linear regression on the rate of decline of dissolved oxygen concentration over the final 4 min of each 5 min respirometer closed cycle period. Data for the period were excluded from analysis when R^2 was below 0.95. The mean recorded levels of background respiration were subtracted and mass-specific $\dot{M}o_2$ stated as g O_2 g^{-1} BW h^{-1}.

2.3.4 Apparent feed intake

Apparent feed intake (AFI) was measured during the feeding experiment and within experimental vessels by procedures similar to that described by Fitzgibbon et al., (2017). Replicate vessels were fed fresh split mussels as described above. Before refeeding, uneaten feed was removed, rinsed with distilled water to remove the salts and frozen at -20^{o}C. Mussel feed intake was measured for all replicate individual rearing vessels and the communal rearing vessel and one control vessel without juvenile lobsters for each rearing treatment to calculate the dry matter weight of feed lost into the water through leaching. The meat of the uneaten feed samples was removed from the shell, oven dried (105^{o}C for 24 hours) and the dry matter weight (DW) measured. The difference between the dry matter weight of feed in control and replicates was used to calculate juvenile feed intake. Apparent feed intake was presented in terms of gram dry matter feed per body weight (gDW individual $lobster^{-1}$ d^{-1}) of lobsters.

Apparent feed intake was determined over three 7-day periods: days 27 to 33, 46 to 52 and 68 to 74. Body weight of all lobsters was measured before and after the completion of each of the three AFI measurements. In each period, the average body weight of the lobsters was 10.20±2.82, 13.31±3.74, 15.60±4.54g in individual rearing and 10.25±2.92, 16.32±5.86, 20.19±6.55g in communal rearing. Individual AFI was only possible in individual rearing replicates whereas in communal rearing only a single mean population AFI was possible.

2.3.5 Calculations and terminology

Growth was measured as body weight gain (ΔW, g), carapace length gain (ΔC, mm) and observed body weight (OW, g) as follows;

ΔW= final body weight, W_i - initial body weight, W_o

ΔC= final carapace length, CL_i - initial carapace length, CL_o

OW= initial body weight, (W_o, g) x body weight gain (ΔW, g)

Biomass was calculated as follow;

Biomass (g) = body weight gain (ΔW, g) x number of individual each treatment

Growth rate was calculated as growth rate of body weight (G_w) and carapace length (G_{CL}) as follows;

$$G_W = \frac{ln\mathrm{Wi} - ln\mathrm{Wo}}{90 \text{ days}}$$

$$G_{CL} = \frac{ln\mathrm{Wi} - ln\mathrm{Wo}}{90 \text{ days}}$$

The coefficient of variation for body weight (CV_W), carapace length (CV_{CL}) growth rate (CV_G), and weight gain (CV_{WG}) were calculated for both initial and final body weight and carapace length for each treatment to examine size disparity (Magnuson, 1962) and growth depensation (McCarthy et al). The CV_W, CV_{CL}, CV_G and CV_{WG} were calculated as:

$$CV_W\,(\%) = \frac{\text{S.D body weight X 100}}{\text{average body weight}}$$

$$CV_{CL}\,(\%) = \frac{\text{S.D carapace lenght X 100}}{\text{average carapace length}}$$

$$CVs_{GR}\,(\%) = \frac{\text{S.D growth rate X 100}}{\text{average growth rate}}$$

$$CV_{WG}\,(\%) = \frac{\text{S.D weigh gain X 100}}{\text{average weight gain}}$$

Apparent feed intake was calculated as follow:

$$\text{AFI (g DM W}^{-1}) = \frac{\text{dry weight feed condumed (g)}}{\text{body weight (g)}}$$

2.3.6 Data analysis

Student's *t*-tests were performed to determine if there were any difference between rearing condition in initial size (body weight and carapace length) and final size, body weight gain, carapace length gain, biomass and daily growth rate. Analyses of co-variance (ANCOVA) were performed to determine if there were any significant differences between rearing conditions in observed weight gain relative to initial body weight. To compare the intermoult period between rearing conditions, a Student's *t*-test was performed. Due to three individuals escaping during the experiment all statistical analyses for individual rearing were performed based on data collected from 17 lobsters.

Residual weight gain (body-weight-corrected) was determined by subtracting the observed ΔW for an individual from that predicted for the lobster by the regression between ΔW and initial body weight for both the individual and communal populations.

The relationship between residual weight gain and sex was evaluated using regression analysis. One-way ANOVA compared the mean residual weight gain between sexes among

treatment groups. To determine if there were any significant difference between treatments in moulting period, Student's *t*-tests were performed.

To explore normality and homogeneity of $\dot{M}o_2$ data, residual plots were used. Boxplots were used to confirm a lack of outliers. Mass-independent data of $\dot{M}o_2$ were expressed as residual for standard, routine active metabolic rates and aerobic scope (rSMR, rRMR, rAMR and rAS, respectively) and calculated from least-square linear regressions for $\dot{M}o_2$ versus body weight as described by Metcalfe et al. (1995). Unlogged plots were used as it was a better fit for the data and is appropriate when using a small mass range of individuals (Metcalfe et al., 1995). Expected $\dot{M}o_2$ for all the 37 lobsters were calculated from the regressions between observed weight gain and initial body weight and residual (body-weight-corrected) metabolic rate were calculated by subtracting the expected $\dot{M}o_2$ from the observed $\dot{M}o_2$.

Least-square linear regressions were fitted to describe the relationship between residual metabolic rates (rSMR, rRMR, rAMR and rAS) and residual weight gain (rΔW). Residual weight gain was used because growth rate and metabolic rate are known to change with body weight (Álvarez & Nicieza, 2005).

The analysis for AFI was performed on the average of the three AFI's measured from the feeding trials for each lobster in individual and the total communal rearing. Student's *t*-test was performed to compare the means of AFI from both rearing treatments. Relationships were compared between residual metabolic rates and AFI using least-square linear regression analysis using IBM SPSS Statistics version 22.0. The level of significance for all analyses was determined at $P<0.05$. Values were presented as mean ± standard deviation (S.E) unless stated otherwise.

2.4 RESULTS

2.4.1 Effects of individual and communal rearing on growth, moulting and apparent feed intake

There was no significant difference in the initial body weight or carapace length of lobsters stocked into the individual and communal rearing treatments (Table 2.1). Following 90 days of rearing, the lobsters in communal rearing were significantly greater in body weight (by 26%) and carapace length (by 7%) compared to those in individual rearing.

The faster growth of communally reared lobsters resulted in a significantly higher body weight gain, carapace length gain, and specific growth rate of body weight and carapace length. (Table 2.1). The final CV_w and CV_{CL} in both treatments were lower after 90 days. However, the lobsters in communal rearing treatment displayed greater size disparity, i.e., higher CV_w and CV_{CL} than those in the individual rearing treatment, and therefore growth depensation. Due to the limited number of experimental replicates, the statistical analysis for the CV could not be performed.

The observed weight gain increased linearly with initial body weight and the relationship was significantly affected by rearing condition (ANCOVA, F=14.461, df 1, 35, P=0.001) (Figure 2.1 and Table 2.2). The slope of the regression was significantly greater for communally reared lobsters due to greater weight gain compared to those in individual rearing. The lobsters in communal rearing displayed greater growth depensation, i.e., larger CV_{WG} than those reared individually.

Expected weight gain for all lobsters was calculated from the regression Table 2.2. The distribution of the residual weight gain was 5-/12+ for individual rearing and 9-/11+ for communal rearing, with minus sign indicating a lobster with higher than expected weight gains, whereas plus sign indicating lower than expected weight gain. Sex did not significantly affect residual weight gain of juveniles in either rearing conditions (Figure 2.2).

Intermoult period was on average reduced in communal rearing compared to that in individual rearing being significantly shorter for the second intermoult period (Table 2.3). The specific AFI in communal rearing lobsters were significantly greater (1.022±0.122%) than those reared individually (0.413±0.040%) (*t*-test, F=4.750, df = 4, P=0.009).

2.4.2 Metabolic rate

The individual metabolic rate **(**SMR, RMR, AMR and AS) of observed $\dot{M}O_2$ increased linearly with body weight (P<0.05) (Figure 2.3 and Table 2.4). The residual metabolic rates were distributed as 16-/21+ for rSMR, 19-/18+ for rRMR, 21-/16+ for rAMR and 20-/17+ for

rAS, with minus sign indicating the number of lobsters with lower than expected metabolic rates and plus sign indicating the number of lobsters with higher expected metabolic rates.. There were significant positive relationships between rΔW and rSMR and rRMR for individual reared lobsters ($P<0.05$) whereas there were no significant correlations for communally reared lobsters ($P>0.05$) (Figure 2.4 and Table 2.5). There were no significant relationships between rΔW and rAMR or rAS for both individual and communal treatments. Residual metabolic rate was not significantly related to AFI in individual reared lobsters (Figure 2.5 and Table 2.6). In communal rearing, analysis of the relationships between metabolic states and AFI was not possible due to an inability to record individual lobster AFI in communal rearing

Table 2.1 Comparison of the growth performance of juvenile lobster *Sagmariasus verreauxi* when reared either individually (n=17) or communally (n=20) over 90 days .

Parameters	Communal	Individual	*t*	df	*P*
Mean initial body weight (g)	5.97±0.62	6.00±0.69	0.030	34	0.76
Mean final body weight (g)	23.47±1.60	18.61±1.20	-2.432	34	0.020*
Mean initial carapace length (mm)	22.42±0.77	22.78±0.82	0.329	34	0.744
Mean final carapace length (mm)	35.92±0.80	33.34±0.72	-2.399	35	0.022*
Body weight gain (g)	17.50±1.12	12.61±0.733	-3.518	35	0.001*
Carapace length gain (mm)	13.51±0.54	10.56±0.47	-4.130	35	0.000*
Biomass (g)	349.94±22.33	214.44±12.46	-5.049	35	0.000*
G_W (%)	0.016±0.001	0.013±0.001	-2.394	32	0.023*
G_{CL} (%)	0.005±0.000	0.004±0.000	-15.702	35	0.000*
CV_w initial (%)	46.63	47.37			
CV_w final (%)	30.40	26.59			
CV_{CL} initial (%)	15.28	14.76			
CV_{CL} final (%)	9.97	8.91			
CV_G body weight (%)	18.24	24.60			
CV_G carapace length (%)	20.68	22.68			
CV_{WG} body weight (%)	28.54	23.96			

* Indicates significant difference (P<0.05).

G_W: growth rate of body weight, G_{CL}: growth rate of carapace length, CV_W: coefficient of variation for body weight, CV_{CL}: coefficient of variation for carapace length, CV_G; coefficient of variation for growth rate, CV_{WG}: coefficient of variation for weight gain.

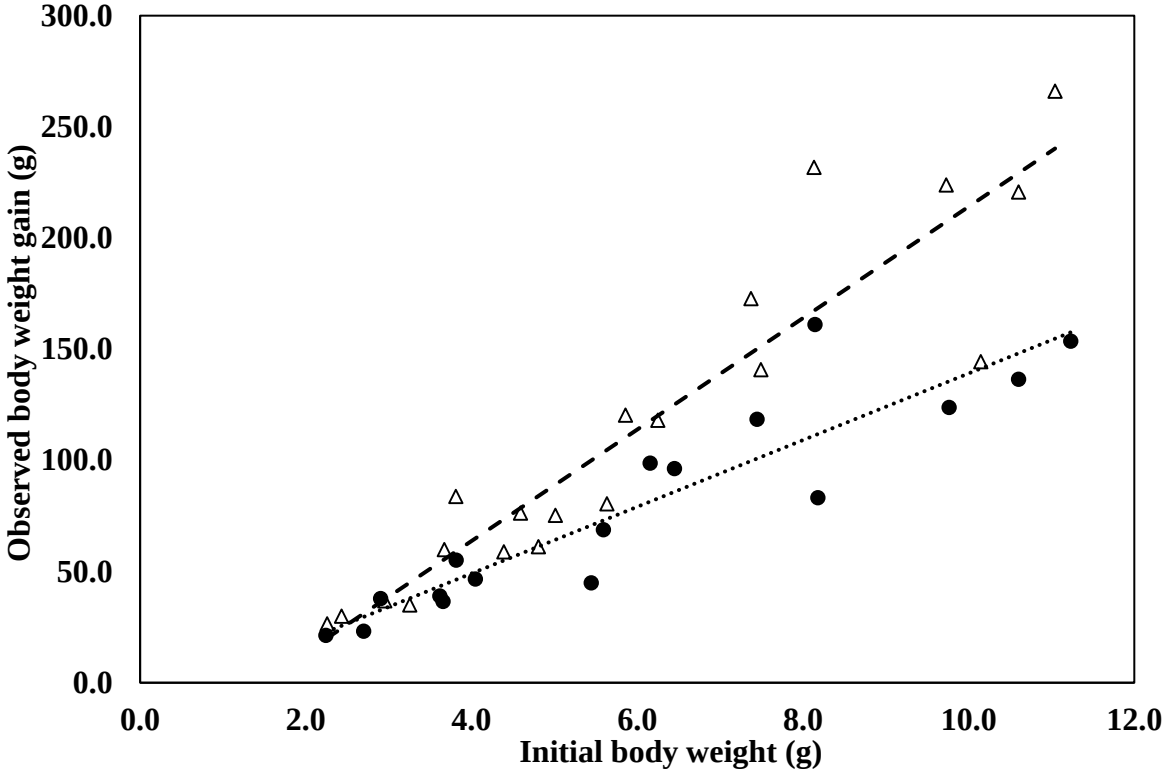

Figure 2.1 Relationship between observed weight gain (ΔW, *g*) and initial body weight (W_I, g) of communal (Δ) and individual (●) reared *Sagmariasus verreauxi* juveniles. Residual (body-mass-corrected) weight gain was calculated by subtracting the expected ΔW with observed ΔW. Each data point represents an individual lobster. Details on regression lines are given in Table 2.2

Table 2.2 Details of linear (y= a+bx) regression describing the relationship between observed weight gain (g) and initial body weight (g) of *Sagmariasus verreauxi* reared communally (n=20) or individually (n=17) over 90 days experiment presented in Figure 2.1

Treatment	a	b	r^2	*P*
Communal	25.058	-36.509	0.8712	<0.05*
Individual	14.996	10.870	0.8478	<0.05*

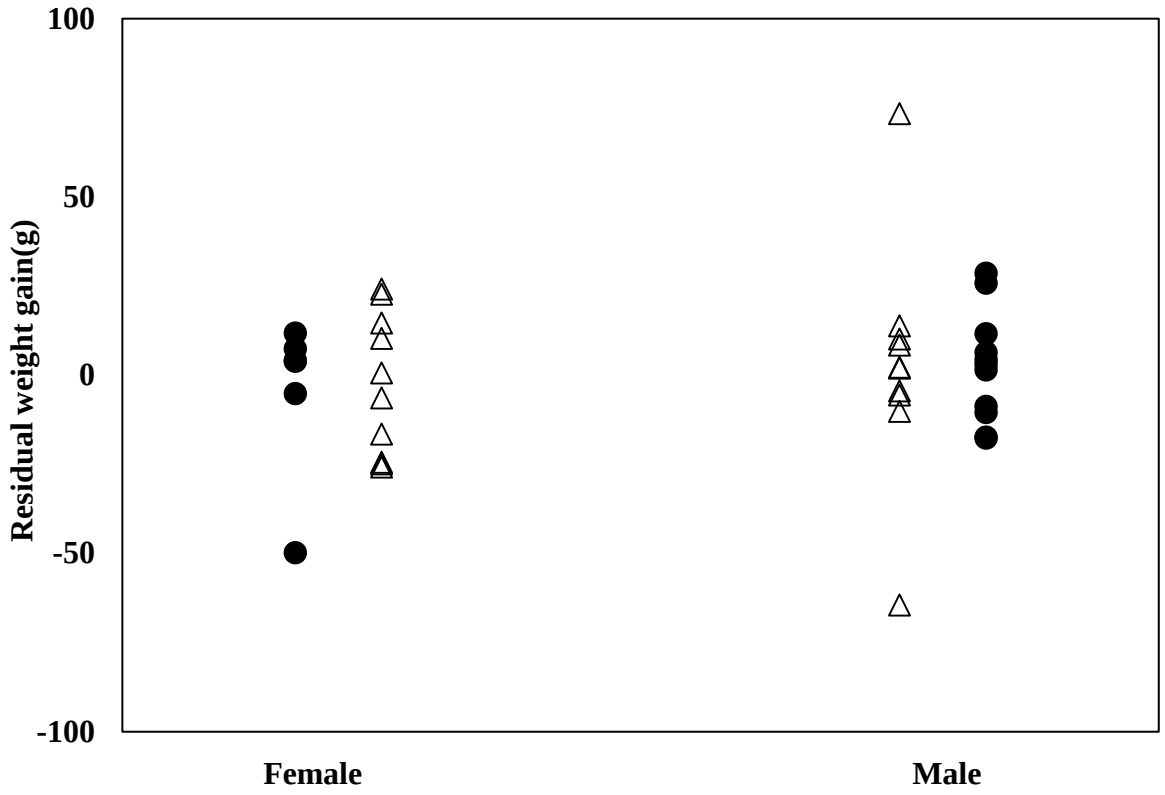

Figure 2.2 Residual weight gain (rΔW, *g*) of female and male *Sagmariasus verreauxi* juveniles reared communally (Δ) and individually (●). One-way ANOVA F=0.584, *P*=0.457 (individual), F=0.173, *P*=0.683 (communal). Each data point represents an individual lobster.

Table 2.3 Comparison of the juvenile lobster *Sagmariasus verreauxi* intermoult period (day) when reared either individually (n=17) or communally (n=20) over 90 days .

	Communal	Individual	t	df`	*P*
1st moult	23.4±0.7	24.2±1.4	0.525	25	0.605
2nd moult	24.8±1.1	31.8±1.5	3.845	31	0.001*
3rd moult	31.5±1.3	34.6±1.8	1.437	31	0.161

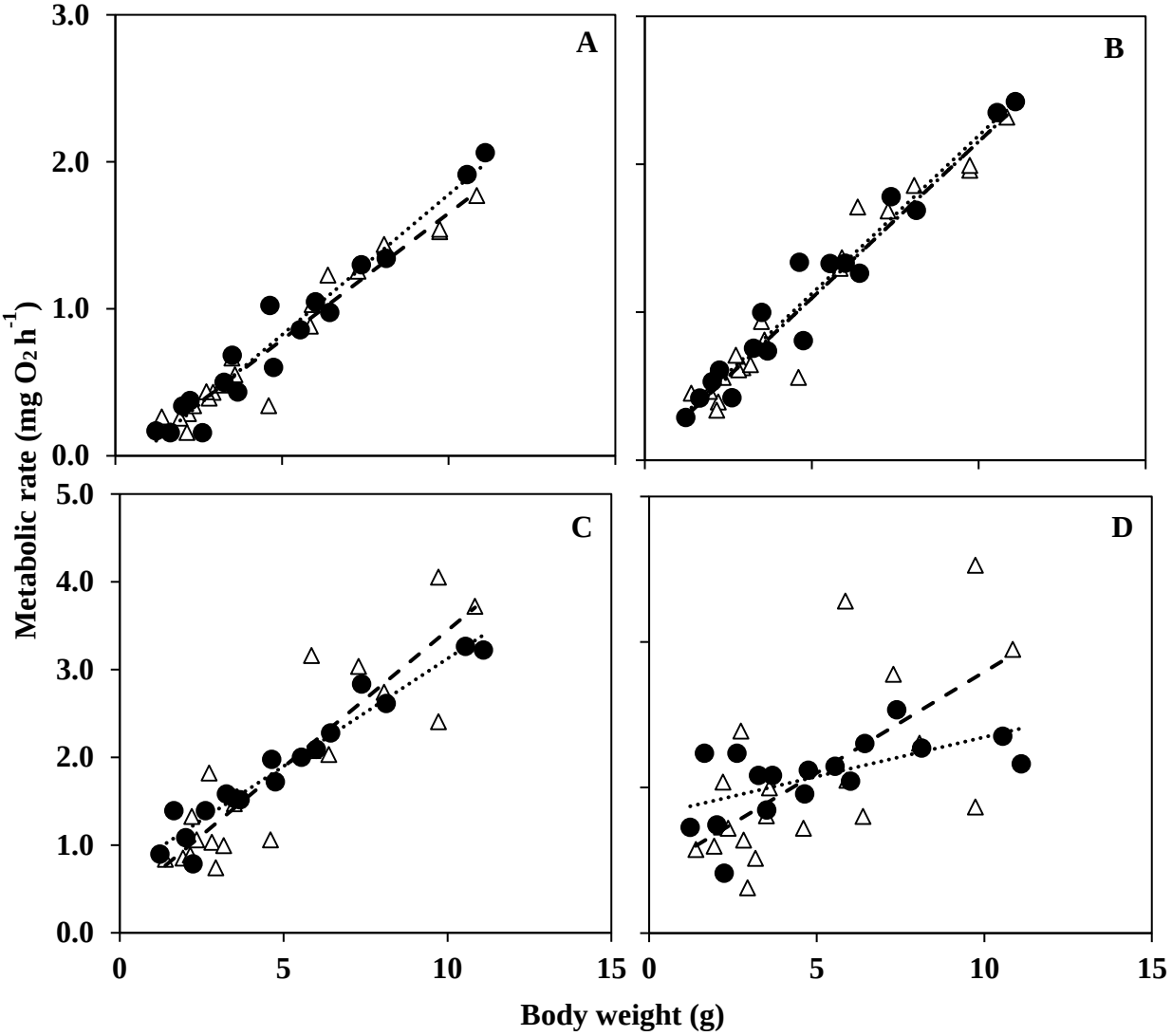

Figure 2.3 Relationship between observed oxygen consumption ($\dot{M}o_2$, mg O^2 h^{-1}) and body weight (g) of *Sagmariasus verreauxi* lobsters reared (●) individually or (Δ) communally. A; standard metabolic rate (SMR), B; routine metabolic rate (RMR), C; active metabolic rate (AMR), D; aerobic scope (AS). Each data point represents an individual lobster. Details of regression lines are presented in Table 2.4.

Table 2.4 Details of linear (y= a+bx) regression describing the relationship between observed standard metabolic rate (SMR), routine metabolic rate (RMR), active metabolic rate (AMR) and aerobic scope (AS) (mg O_2 h^{-1} g^{-1}) and body weight (g) of *Sagmariasus verreauxi* lobsters presented in Figure 2.3 (ANOVA, P <0.05).

Treatment	Metabolic rate (mg O_2 h^{-1})	a	b	r^2	*P*
Individual	SMR	-0.131	0.191	0.956	<0.001
	RMR	0.057	0.214	0.953	<0.001
	AMR	0.674	0.245	0.942	<0.001
	AS	0.8051	0.054	0.351	0.012
Communal	SMR	-0.073	0.172	0.946	<0.001
	RMR	0.036	0.218	0.941	<0.001
	AMR	0.333	0.311	0.794	<0.001
	AS	0.406	0.139	0.444	0.001

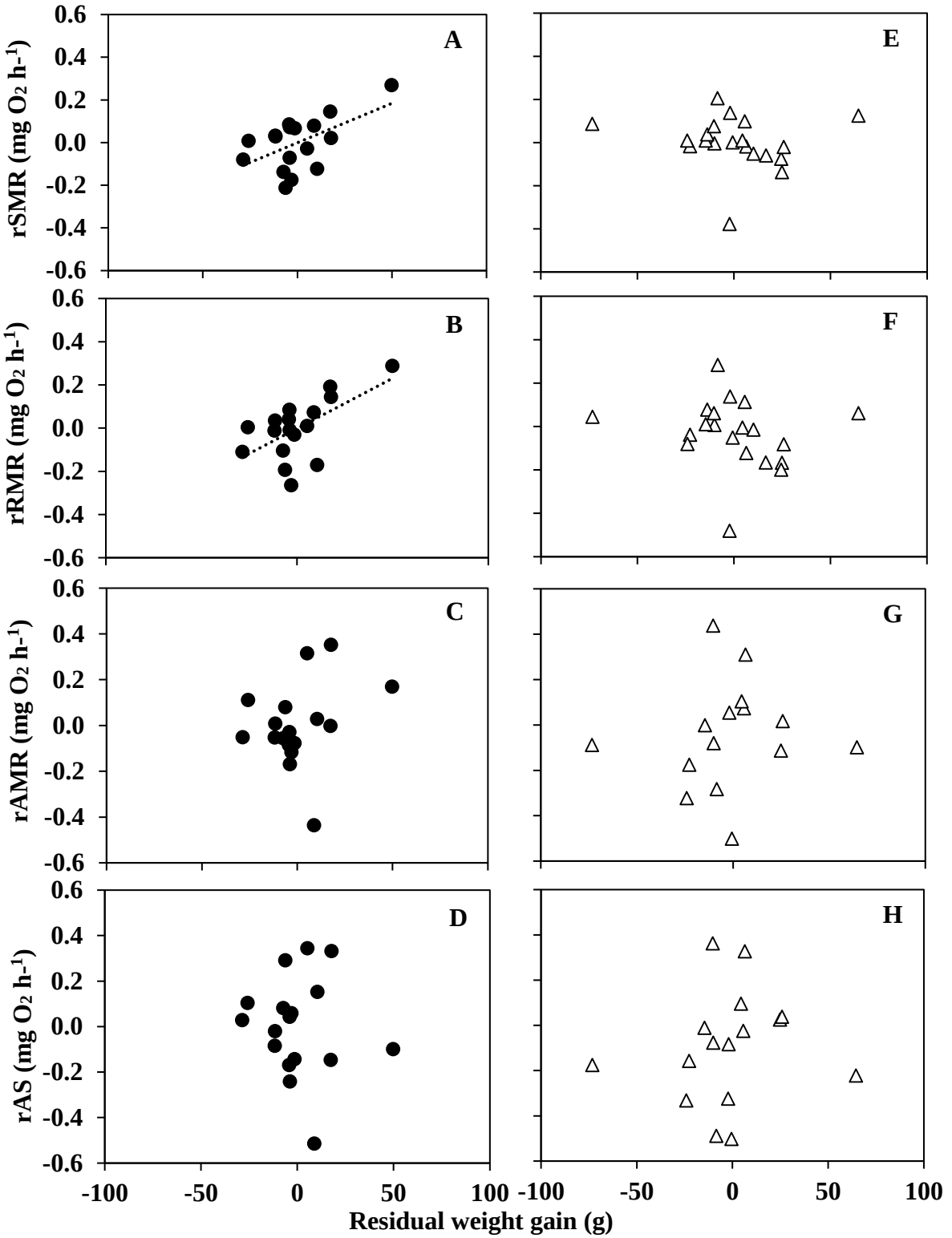

Figure 2.4. Relationship between residual mass gain (ΔW, g) with residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Sagmariasus verreauxi* lobsters reared individually (A-D) or communal culture (E-H). Details on regression lines (dotted lines) are given in Table 2.5.

Table 2.5 Details of linear (y= a+bx) regression describing the relationship between residual weight gain (rΔW, g) and residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Sagmariasus verreauxi* reared communally or individually over 90 days experiment presented in Figure 2.4.

Treatment	Metabolic rate (mg O_2 h^{-1})	a	b	r^2	*P*
Individual	SMR	-0.001	0.004	0.298	0.024
	RMR	-0.002	0.005	0.357	0.011
	AMR	0.001	0.003	0.065	0.323
	AS	0.001	-0.001	0.009	0.722
Communal	SMR	0.002	-0.001	0.015	0.603
	RMR	-0.030	-0.001	0.031	0.455
	AMR	0.001	0.001	0.005	0.784
	AS	0.001	0.002	0.010	0.676

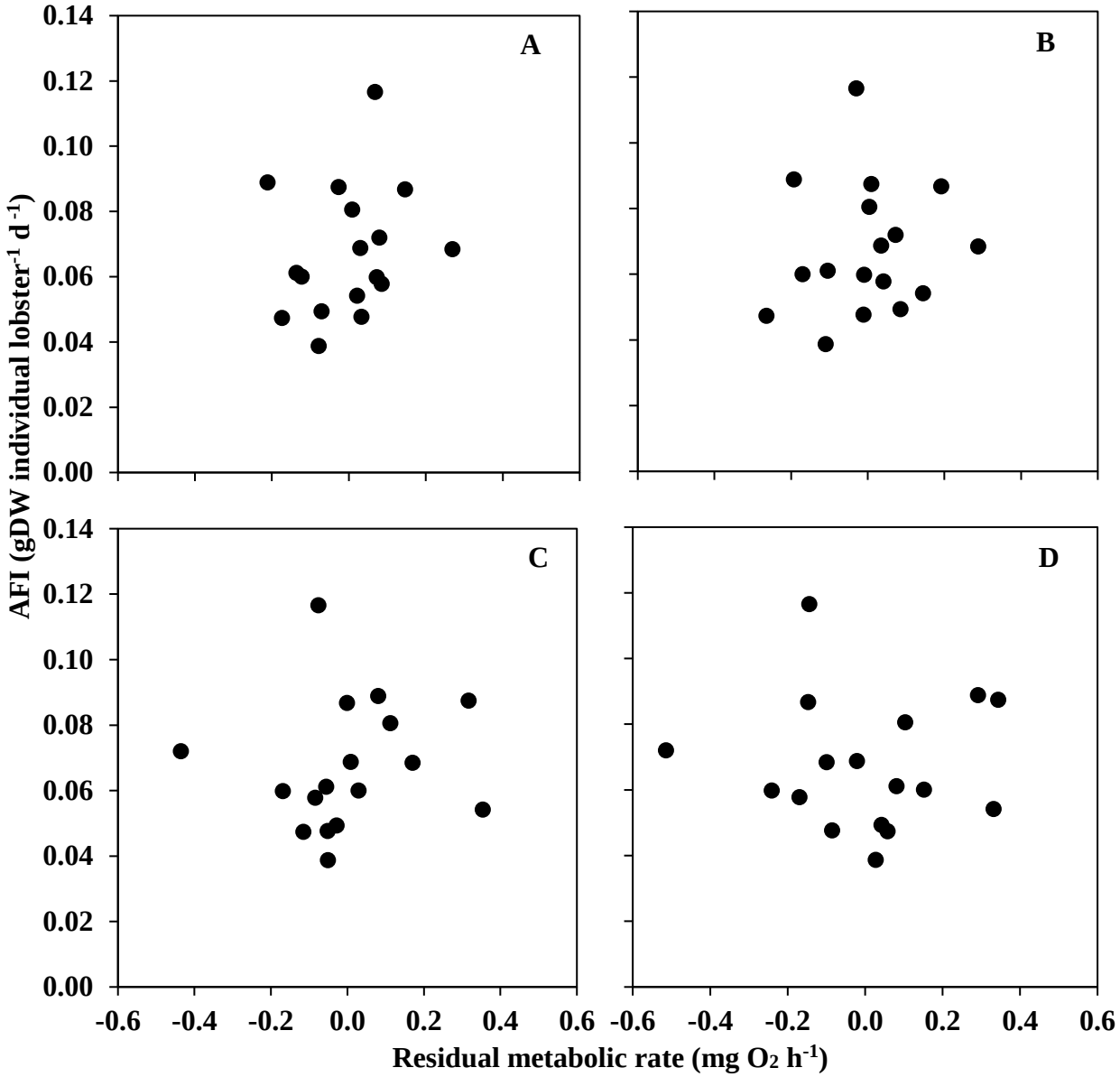

Figure 2.5. Relationship between apparent feed intake (AFI) and residual metabolic rate; (A) residual standard metabolic rate (rSMR); (B) residual routine metabolic rate (rRMR); (C) residual active metabolic rate (rAMR) and; residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Sagmariasus verreauxi* lobsters reared individually over seven days feeding experiment. Each data point represent an individual lobster. Details of linear regression are presented in Table 2.6.

Table 2.6 Details of linear regression (y=a+bx) describing the relationship between individual residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) (mg O_2 h^{-1}) and apparent feed intake (AFI) of *Sagmariasus verreauxi* reared individually (n=17) over seven days feeding experiment presented in Figure 2.5.

Metabolic rate	a	b	r^2	*P*
rSMR	0.067	0.035	0.047	0.403
rRMR	0.067	0.018	0.017	0.615
rAMR	0.067	0.013	0.014	0.647
rAS	0.067	-0.002	0.001	0.933

2.5 DISCUSSION

This present research is the first study to examine the relationship between metabolic phenotype and growth performance of spiny lobsters. Standard and routine metabolic rate in individual rearing was significantly related to growth in *S. verreauxi* juveniles indicating there is a direct link between aerobic metabolic status of individuals and growth in the absence of social interaction. In contrast, the same relationship between metabolic rate and growth was not observed in communal rearing demonstrating an overriding influence of social interaction on lobster growth. These results suggest that growth performance of spiny lobsters can be linked with individual metabolic status but social behaviour can play a more important role in determining the growth of a cohort.

2.5.1 Effects of individual and communal rearing on growth, moulting and feeding activity

Rearing treatments had a significant effect on growth, moulting and feeding activity in *S. verreauxi* juveniles. Lobsters in communal rearing grew significantly faster and had greater size disparity and growth depensation than those in the individual rearing treatment. This is consistent with results from *Panulirus homarus* where juveniles (90- 200g) grew three times faster in communal rearing (Vijayakumaran et al., 2010). Similarly, lobsters (2-3g) of *Panulirus ornatus* also grew 40% faster in communal rearing compared to lobsters reared individually (Irvin & Williams, 2008). Irvin and Williams (2008) suggested that higher growth rate in communal rearing was due to cannibalism and the stimulation of feeding by the competition for food with conspecifics. Agonistic behaviour and the ability to form highly-structured dominance hierarchies has been observed among spiny lobsters (Fielder, 1965a ; Fielder, 1965b ; Cobb, 1981 ; Thomas et al., 2003 ; Segura-García et al., 2004). The presence of dominant individuals, can directly or indirectly suppress the growth of subordinates by promoting direct competition for food, appetite suppression, altered food-conversion efficiency and an increase in motor activity in the subordinate individual (Karplus, 2005). In spiny lobsters, Thomas et al. (2003) reported agonistic behaviour where the dominant individual controlled a disproportionate share of food resources compared to subordinates which caused size disparity and growth depensation. In the present study, the CV_{WG} was greater in communal rearing indicating that culturing the lobsters communally appeared to increase the growth depensation in early stages of juvenile development. However, according to Carter et al. (2014), study shown by Simon and James (2007) demonstrates that *Jasus edwardsii* adults did not show any growth depensation when reared over 8 months period which indicates that the

growth depensation of *J edwardsii* may reduce with age and adequate feeding. No mortality or cannibalism in communal rearing were recorded in the present study demonstrating that improved growth in communal rearing was not due these factors.

Intermoult period was significantly shorter in communal rearing lobsters than those in individual rearing over the second moult. This finding is consistent with results from *P. homarus* and *P. ornatus* where the moulting frequency of the communal lobsters were higher than those reared individually. Lobsters are reported to have the ability to recognise the odour of conspecifics such as chemical stimuli produced during moulting, agonistic and reproductive behaviours (Aiken and Waddy, 1980; Waddy and Aiken, 1990). It is possible that a chemical stimulus from moulting conspecifics is important for promoting moulting (Vijayakumaran et al., 2010) and this could explain the longer moulting period of individually reared lobsters in the present study.

Lobsters in communal rearing displayed higher AFI which would have contributed to improved growth. The social interaction in the communal rearing may have provided triggers or cues to stimulate the lobsters feeding possibly in response to competitive interactions. (Karplus, 2005 ; Irvin & Williams, 2008). Spiny lobsters are known as highly gregarious animals and often collect in dense aggregations in both wild and rearing environments (Fielder, 1965b; Berrill, 1976; James et al., 2001; Moyle et al., 2009). With the presence of conspecifics, this may be important for controlling stress and promoting optimum feeding and growth. In controlled experiments, there is clear evidence of social interaction influencing growth in crustaceans (Karplus & Barki, 2004).

2.5.2 Metabolic rate, growth and feed intake

Metabolic rate had a significant relationship with the growth rate of individually reared *S. verreauxi* lobsters but not communally reared lobsters. The growth rate variances in individual rearing lobsters are possibly linked to variability in the metabolic physiology of individuals and nutrient assimilation (Millidine et al., 2009). This finding is consistent with results shown from previous studies on fish where individual variation in metabolic rates has been linked specifically with growth; i.e. fish with a relatively higher metabolic rate can have more rapid growth in high feed environments (McNab, 1980 ; McNab, 1986 ; McCarthy, 2000 ; Álvarez & Nicieza, 2005). This is despite higher metabolic rate animals having a higher cost of maintenance which requires higher rates of food intake to maintain larger 'metabolic machinery' and greater potential processing food (Millidine et al., 2009 ; Biro & Stamps, 2010). Standard metabolic rate is expected to have a positive effect on the animal fitness under

the ‘increased intake’ hypothesis (Burton et al., 2011). Based on this hypothesis, lobsters with higher metabolic rates can take advantage of greater excess of resources which can be directed to other functions such as growth after accomplishing individual’s daily expenditure.

In communal rearing, social interaction outweighed the direct relationship between metabolic rate and growth. This may be due to high SMR lobsters not benefiting from the ‘increased intake’ hypothesis as observed in individual rearing lobsters due to the energetic cost of being dominant (Røskaft et al., 1986 ; Bryant & Newton, 1994). Therefore, it may give an advantage to the lower metabolic rate juveniles by allowing them to grow as fast as the dominant in the communal rearing as mentioned in the ‘compensation’ hypothesis where greater excess of resources can be directed towards growth (Burton et al., 2011). There are numerous conditions which might negate the growth advantage of the high metabolic rate animals in communal rearing including the influence of sex, dominance and competitive ability, (Brown et al., 2003 ; Álvarez & Nicieza, 2005 ; Killen et al., 2013). The result of the present study shows no interaction between growth and sex in either rearing condition. This is possibly due to the immature status of lobsters examined. Previous studies of aquatic organisms show that high SMR animals can be highly aggressive (Metcalfe et al., 1995 ; Cutts et al., 2001 ; Reid et al., 2011 ; Reid et al., 2012). Intrinsic aggressiveness can also be linked with the risk of injury and major costs in terms of either energy expended during agonistic contests or loss of feeding opportunities (Turnbull et al., 1998 ; Nicieza & Metcalfe, 1999). Therefore, the costs of being aggressive can indirectly generate a non-significant relationship between metabolic rate and growth as observed in the present study.

The result of the present study shows no association between metabolic rate (SMR, RMR, AMR and AS) and AFI of spiny lobster in the individual rearing. This finding is in contradiction with the previous studies on brown trout, *Salmo trutta* higher metabolic rate *S. trutta* exhibit greater food consumption to cover the high cost of maintenance (Auer et al., 2015a ; Auer et al., 2015b). The absence of a relationship between metabolic rate and individual AFI in the current study suggests that higher metabolic rate lobsters may not require greater amounts of food to uphold their larger maintenance cost. This could be due to having greater digestive and assimilation efficiency compared to lower metabolic rate lobsters. Earlier studies on teleosts revealed that individual conversion efficiency is highly correlated with physiological and anatomical traits and feed intake (Trudel et al., 2001 ; Metcalfe, 2016a ; Allen et al., 2016 ; McCarthy et al., 1993 ; Carter et al., 1994). Studies on rainbow trout displayed that fish growth rates are not linked with their metabolic phenotype, but associated with the size of gastrointestinal tracts, maximum feeding capacity and growth efficiency (Allen

et al., 2016). Furthermore, the findings of the present study may also be attributed to the probability of inaccuracies in AFI measurement which is known to have inherent imprecisions in studies with crustaceans due to their messy feeding habits (McGaw & Penney, 2014). Spiny lobsters are defined as "messy feeders" where food is manipulated externally into smaller particles before consumption by tearing, pulling and grinding using their mouth parts (Guillaume & Ceccaldi, 2001).

2.5.3 CONCLUSION

The results of this present study demonstrated that growth performance of *S. verreauxi* is correlated with metabolic phenotypes but social behaviour plays a more dominant role in determining the growth of individuals. High metabolic rate lobsters grew significantly faster than low metabolic rate lobsters when reared individually. However, with the association of social interaction, no correlation between energy metabolism and growth were observed in communally reared juveniles. Social interactions may cause the lobsters to use their individual energy for agonistic interactions that could otherwise be used for other physiological functions, such as growth. Communally reared lobsters grew faster and had greater feed intake than individually rearing. This is possibly due to feeding competition with conspecifics, which also resulted in greater growth disparity and depensation in communal rearing. Metabolic rate can be used as a reliable predictor for spiny lobster's growth rate in individual culture. However, understanding the correlation between metabolic phenotype and social behaviour (dominance and aggression) of spiny lobster requires further investigation.

CHAPTER 3

THE INFLUENCE OF SIZE, SEX, METABOLIC PHENOTYPE, EXPERIENCE AND REARING HISTORY ON SOCIAL DOMINANCE STATUS OF SPINY LOBSTER IN CULTURE

3.1 ABSTRACT

Social behaviour plays an important role in determining the growth of spiny lobsters in captivity. Competition in a population of social animals for limited resources, such as food, is an important factor contributing to growth of individuals. An animal's ability to compete for resources is often determined by the combination of its morphological and physiological characteristics such as body size, sex and energy reserves as well as prior experience. The present study examined the effects of lobster size, metabolic phenotype, sex, feeding contest experience and rearing history on the social dominance status (dominant, neutral or subordinate) of spiny lobster *Sagmariasus verreauxi* juveniles in captivity. Thirty-three intermoult lobsters with a mean carapace length (CL) of 48.2±5.0 mm for males (n=18) and 50.0±6.2 mm for females (n=15) were used in a series of randomly paired feeding contest experiments over 3- day periods of observation. Competitive ability and dominance displayed by the lobsters were determined by recording orientation, avoidance (no response or retreat), attacking (fighting) and feeding behaviours. Size was an important predictor of spiny lobster social dominance status. Moreover, social dominance status was significantly linked with size, metabolic phenotype and sex. Larger lobster was predicted to be dominant over smaller size lobster and, female lobster showed potential to become more dominant than male lobster irrespective of size and metabolic phenotype status. Disparate from findings with fish and other crustacean species, low metabolic rate lobsters displayed a greater ability to win over high metabolic rate lobsters. These findings demonstrate that a combination of individual lobster body size, metabolic phenotype and sex can be used as consistent predictors of social dominance status in spiny lobsters. Consequently, this study suggests that specific morphological and physiological traits have a crucial role in determining spiny lobster social behaviour status and therefore growth performance.

Keywords: Dominance status, social behaviour, morphological and physiological traits, spiny lobster, growth

3.2 INTRODUCTION

In a population of a social species, intraspecific competition for valuable and limited resources such as food and space often arises (Yamamoto et al., 1998). Resulting aggression and conflict often play important roles in determining structure and distribution of an individual animal in a group or population such as formation of social dominance hierarchies (Barnard & Burk, 1979 ; Chase et al., 2002). An individual's status and access to food and shelter can be defined from social dominance hierarchies where the higher the social status, the more advantaged the individual. The dominant individual wins most of the encounters over the subordinate individual, which in turn loses the majority of the encounters and retreats (Tattersall, 2012).

The dominance status and intraspecific competitive ability in aquatic species are strongly related to factors such as differences in size, sex, moulting stage, availability of feed, recent agonistic experience and energy metabolism (Fielder, 1965a ; Fielder, 1965b; Metcalfe et al., 1995 ; Karavanich & Atema, 1998 ; Thomas et al., 2003 ; Cobb & Phillips, 1980). Determining the characteristics of individual animals that influence the outcomes of agonistic interactions is therefore important for predicting the individual animal's social status and growth performance.

In most crustaceans, size and sex are important factors in determining the outcome of encounters: larger males are generally more aggressive and tend to dominate over smaller conspecifics, often monopolizing resources such as food and shelter (Fielder, 1965a ; Fielder, 1965b; Lee & Fielder, 1983 ; Pavey & Fielder, 1996 ; Issa et al., 1999 ; Beattie et al., 2012 ; Briones-Fourzán et al., 2014). In the spiny lobster *Jasus edwardsii*, Thomas et al. (2003) demonstrated that larger lobsters are regularly able to maintain their size status when feeding opportunities are more restrictive but as feed availability increases, fewer of the initially largest size-ranked lobsters maintain their status. Metabolic phenotype has also been reported by Brown et al. (2003) to be one of the factors in influencing giant freshwater prawn, *Macrobrachium rosenbergii* dominance status where individuals with higher energy metabolism were found to be more aggressive and dominant than those that become subordinates.

Spiny lobsters generally display complex social behaviour, being gregarious in nature and living and often sheltering with conspecifics in both the wild and in captivity (Berrill, 1975 ; Herrnkind, 1980 ; Cobb, 1981). Early work reported that spiny lobsters exhibit aggressive and complex social behaviours which they use for the development of social hierarchies (Shabani et al., 2009). Social hierarchical structure and agonistic behaviour displayed by

dominant individuals helps them control a disproportionate share of food resources compared to the subordinates. This has been stated as one of the factors that contributes towards growth disparity and depensation in spiny lobster rearing (Thomas et al., 2003 ; Carter et al., 2014).

Previous research by Irvin and Williams (2008) and Vijayakumaran et al. (2010) demonstrated that with the association of social interaction in communal rearing, *Panulirus ornatus* and *Panulirus homarus* juveniles displayed a greater growth rate and growth disparity than individually reared juveniles. Similar patterns have been also observed in *S. verreauxi* (Chapter 2). Improve growth performance in communal rearing is thought to be linked to greater feed intake in comparison to individual rearing. This was probably due to social interaction with conspecifics such as feeding competition. Feeding competition may be beneficial for some individuals because it can improve growth. In contrast, competition for food might also reduce growth of some individuals. Consequently, there will be greater growth disparity and therefore growth depensation in communal rearing. Additionally, results from the previous chapter also demonstrated that growth performance of spiny lobsters was also found to be correlated with individual variation in metabolic phenotype when cultured individually where higher metabolic rate lobsters demonstrated greater growth rate than lower metabolic rate lobsters. However, with the association of social interaction there was no correlation found between metabolic phenotype and growth. Therefore, gaining further insight into the links with metabolic phenotype and social behaviour (dominance and aggression) is required to understand the apparent disconnect between energy metabolism and growth in communal culture.

The aim of this study was to examine the influence of morphological and physiological traits, rearing condition and prior experience on the dominance status and feeding competitive behaviour of spiny lobster *S. verreauxi* juveniles in paired feeding contests. The morphological traits were, size and sex and the physiological traits were, metabolic phenotype. Understanding the effects of these influences on social dominance status and feeding competitive behaviour has the potential to identify the cause of the disconnect between energy metabolism and growth in communal rearing and growth performance of individual lobsters in captivity

3.3 MATERIALS AND METHODS

3.3.1 Experimental animals

A total of 33 juvenile lobster, 18 males (individually cultured (I) =7, communal cultured (C) =11) and 15 females (I=3, C=12) with the carapace length (CL) (measured from the base of the eye socket to the posterior-medial edge of the cephalothorax) of 48.2±5.0 mm for males and 50.0±6.2 mm for females were examined in paired feeding contest experiments. All the lobsters in this experiment were reused from the previous experiment and held as described in Chapter 2 prior to feeding contest experiments. Only intermoult lobsters (7-15 days post moult) were used to avoid the potentially confounding effects of moult cycle (Atema & Cobb, 1980 ; Lipcius & Herrnkind, 1982 ; Peeke et al., 1998).

3.3.2 Paired feeding behavioural experimental design

Four experimental units were used in this experiment, each unit consisted of a rectangular high-density polyethylene vessel (42.5 cm length, 27.5 cm width, and 24.5 cm height, filled with 28 L of filtered seawater) with water flowing through the water outlet from the other end at a rate of three exchanges per hour (Figure 3.1). A video camera (GoPro Hero 3) was placed on top of the tank to record the lobsters' behaviours. The experiment was conducted inside a dark room to encourage normal nocturnal feeding behaviour with dim red light from a fluorescent globe (Sylvania, 36W red colour) used to provide sufficient illumination for video recordings without disturbing normal behaviour of the lobsters as lobsters cannot perceive red light (Weiss et al., 2006). Two cylindrical shelters and a feeding tray were provided in each experimental unit. Throughout the experiment, the water temperature was maintained at 21^{o}C.

3.3.3 Paired contest protocol

To determine whether lobsters were dominant, neutral or subordinate, a pairwise win-loss analysis of interactions conducted (Pavey & Fielder, 1996). Two randomly selected lobsters were placed in each of the experimental units and were left undisturbed for 24 h to acclimatize (Figure 3.2). Each member of a pair was selected based on the following criteria; (1) had a full set of appendages and no injury, (2) were returned to their original rearing vessels after previous feeding contest experiment and reared for a minimum of three days, (3) they had not previously been opponents. During the acclimation period, food was not provided. Over the 3-day period of observation, the ability of the two juveniles to compete for the food items was recorded, together with the incidence of behaviour interactions (Yamamoto et al., 1998).

In each pairing, a single split fresh blue mussel (*Mytilus galloprovincialis*) (ca. 3-5g with shell) was placed into the feeding tray once a day between 09:00 and 10:00. A single mussel was less than the amount that could be consumed by a single lobster in a day (determined from previous observations made on individually reared lobsters). Contests were considered to have begun when the lobsters oriented to the food item and ended when the food item was completely ingested. The empty shell was removed at the end of each observation period. Feeding and observation protocol for each pair was repeated for three consecutive days.

These measurements of behaviour are referred to as competitive ability and was assessed using two measures: the feeding behaviour in order to obtain food and the feeding success (Table 3.1). Dominance status (dominant, neutral or subordinate) was assessed on the competitive ability obtained from the feeding behaviour and success score. Feeding behaviour and success scores were calculated over the three-day observational period which produced three contest records for each lobster. Lobsters was determined to be dominant when they won two or three contests otherwise, the lobsters were ranked as subordinate. When both lobsters shared a food item on two or three occasions, both lobsters were ranked as neutral.

Overall a total of 150 contests (50 pairs x 3 observations) were carried out using 16 experimental units. At the end of any observation experiment, both lobsters were returned to their original rearing vessels and reared for at least three days before being potentially reused and introduced to a new partner. Experimental vessels were drained and thoroughly brushed and disinfected to ensure that no lobster odours remained before the introduction of a new pair of lobsters (Briones-Fourzán et al., 2014). In the experiment, 29 lobsters were randomly selected and reused to determine if prior feeding contest experience influenced the competitive ability of the lobsters (Table 3.2). To investigate the relationship between sex and dominance status, 3 pairing sex combinations were made: male-male (14 pairs), female-female (11 pairs) and male-female (25 pairs).

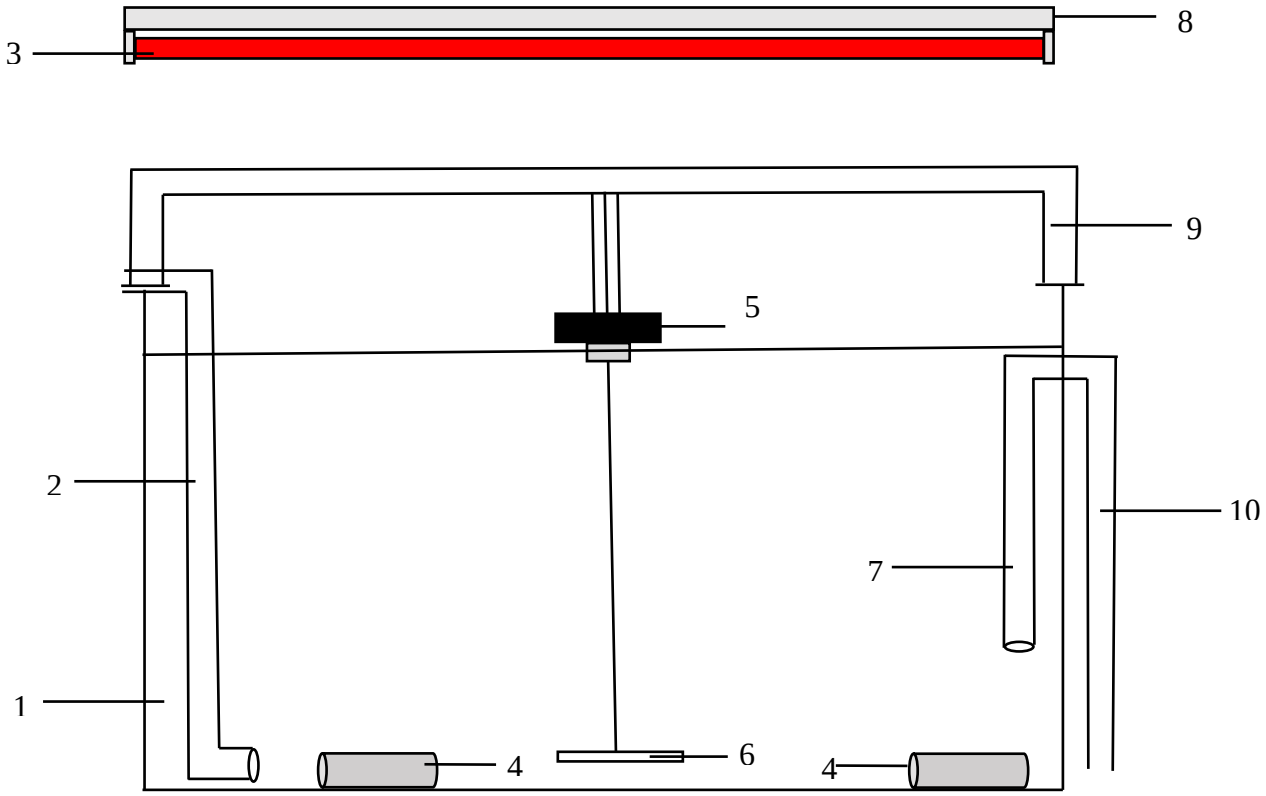

Figure 3.1 The experimental unit used for the feeding behaviour experiment. 1, Experimental tank; 2, in-flow tube; 3, fluorescent globe; 4, shelter; 5, camera; 6, feeding tray; 7, out-flow tube; 8, globe holder; 9, camera stand.

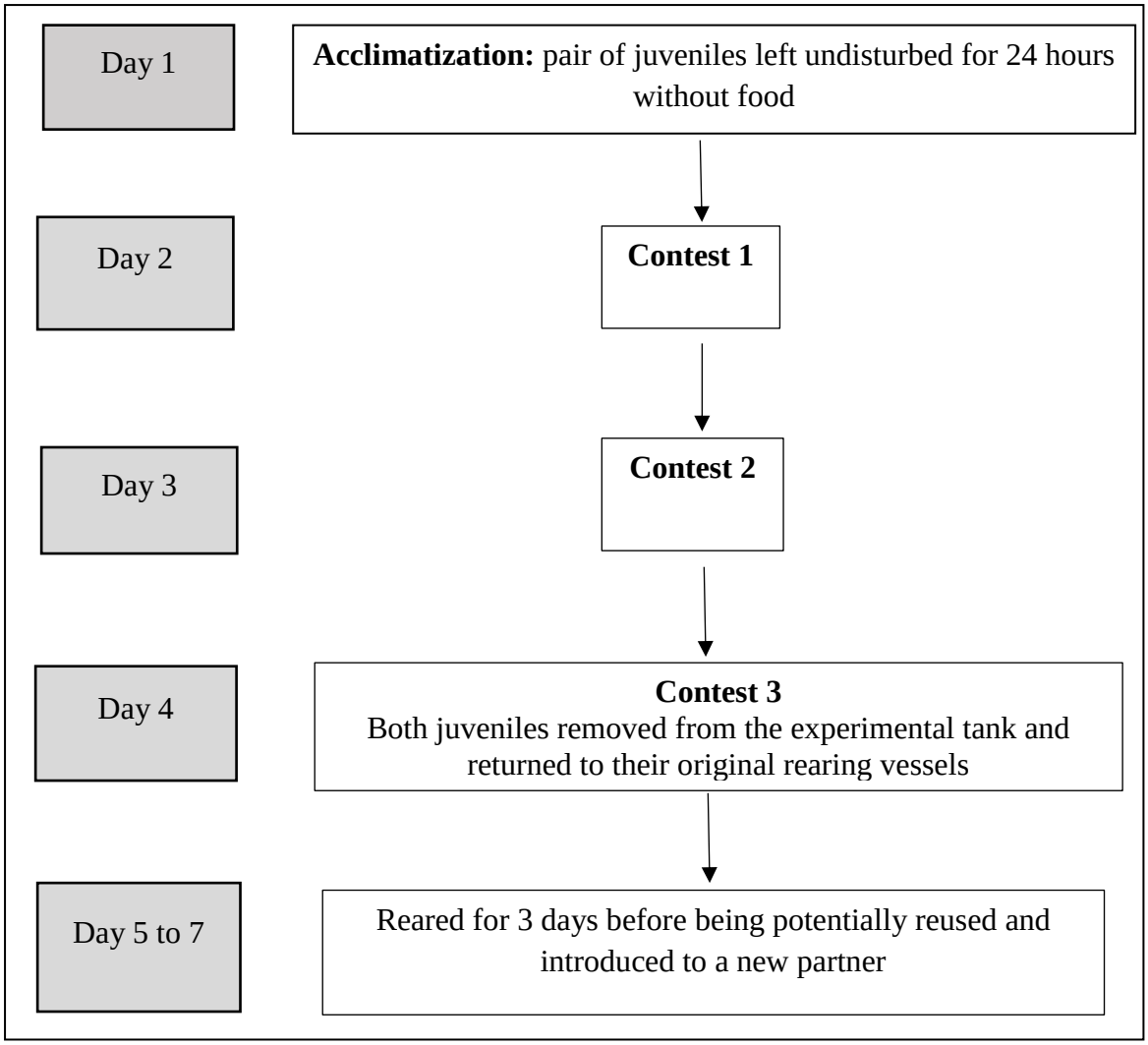

Figure 3.2 A diagram showing the feeding experimental procedure.

Table 3.1 The protocol used to score the feeding behaviour and success of the lobster in each pair (modified from McCarthy, 2001 ; Carter et al., 2014 ; Briones-Fourzán et al., 2014)

Dominance Status (Score)	Behaviour Score	Definition	Description
Subordinate (-1)	1	No response (NR)	Lobster did not show any response to the food item.
	2	Orientation (O)	Lobster searched and moved toward the food item but did not contest for the food.
	3	Secondary consumer (C1)	Lobster approached, captured (grasped and clasped) and ingested the food item only after the rival finished ingesting the food item.
	4	Fight and lost (FL)	Lobsters attempted capture (grasped and clasped) and fought for the food item but lost the contest with the other lobster for the food item and retreated from the other lobster.
Neutral (0)	5	Share (S)	Both lobsters captured (grasped and clasped) and ingested the food item at the same time.
	6	Fight and win (FW)	Lobster captured (grasped and clasped) and fought for the food item and won the contest for the food.
Dominant (+1)	7	Primary consumer (C2)	Lobster approached, captured (grasped and clasped) and ingested most/all the food item without needing to contest with the other lobster.

Table 3.2 Summary of feeding contest experience and number of lobsters that were reused in all experiments (n=50)

No. of feeding contest experiences	No. of lobsters
1	4
2	6
3	13
4	6
5	3
6	1

3.3.4 Oxygen consumption rate ($\dot{M}o_2$)

Before commencement of the rearing experiment in Chapter 2, the oxygen consumption rate ($\dot{M}o_2$) of all lobsters was measured using automated intermittent flow-through respirometry similar to that described by Fitzgibbon et al. (2014). The respiratory system comprised of four 50 ml polypropylene respiratory chambers (50 ml conical bottom centrifuge tubes) with internal diameter of 30 mm and a length of 115 mm submerged in a water bath (24 cm height, 24 cm length, 25 cm width) that received seawater continuously at 17 l per hour from a 500 l insulated sump which was heated to the treatment temperature (21±0.2^{o}C) by an emersion heater. Two twin channel mini peristaltic pumps (Harvard MP II mini-peristaltic pump) were used to continuously circulate water at a rate of 10 ml min^{-1} through the chambers and past an oxygen sensor. Dissolved oxygen inside the chambers was recorded and logged every 20 s by a fibre optic oxygen microsensor meter (OXY- 4 mini, www.preSense.de) and connected with a computer. Another two-twin channel peristaltic pump was used to introduce new water from the external water bath at the rate of 14 ml per min. A digital recycler timer (Sentinel DRT-1) was connected to the pumps and was programmed to turn on in 10 min on and 5 min off cycles. Each chamber was alternated between closed and flow-through cycles which allowed a $\dot{M}o_2$ measurement every 15 min, air delivered through an aquarium air stone maintained the dissolved oxygen concentration in the external water bath at 100% saturation. A black corflute screen was used to enclose the water bath housing in order to exclude light and external stimuli. Dissolved oxygen within the respiratory chambers never fell below 85% throughout the $\dot{M}o_2$ measurements. The respiratory system was sterilized with a 1 mg per l solution of sodium hypochlorite, rinsed with fresh water, and air dried following each experiment.

Lobsters were starved for 24 hours to clear the digestive tract of food and faeces and eliminate variability of measurements associated with thermic effect of food (total energy expenditure above the SMR due to the cost of processing food for use and storage) or also known as specific dynamic action (SDA) before $\dot{M}o_2$ measurement. In the late evening after 24 h of starvation, individual lobsters were placed into the respirometer chambers and $\dot{M}o_2$ logged overnight for 16 h for approximately 64 $\dot{M}o_2$ measurements. The mean of the lowest five recordings of the $\dot{M}o^2$ recorded was defined as standard metabolic rate (SMR) and the average of all 64 recordings was defined as routine metabolic rate (RMR). To stimulate active metabolic rate (AMR), each lobster was removed from the respirometer and made to swim by encouraging the lobster by hand to swim inside a small tub (40 cm height, 60 cm length, 30 cm width) until it became exhausted and non-responsive to stimuli (approximately 10 min).

Lobsters were then placed back into the chamber and $\dot{M}o_2$ recorded for 2 h. The exhaustion protocol was maintained to keep in time with the open cycle of the respirometer system to allow immediate $\dot{M}o_2$ measurements. The mean of the highest 10% recording of the oxygen consumption rate measured after the exhaustive exercise was defined as active metabolic rate (AMR). Aerobic scope (AS) was determined by subtracting the SMR from AMR. All lobsters were removed from the chamber immediately after respiratory measurement completed and wet body weight (BW) was recorded after drying the lobster with paper towel. Oxygen demand of the respirometer system was then recorded for another 1 to 2 h as a measurement of background respiration. Oxygen consumption rates were expressed in mg O_2 h^{-1} BW^{-1} after the subtraction of background measurements obtained from empty chambers.

Lobster $\dot{M}o_2$ were determined using linear regression to the rate of decline of dissolved oxygen concentration over the final 4 min of each 5 min respirometer closed cycle period. Data for the period were excluded from analysis when the linear regression coefficients were R^2 below 0.95. The mean recorded levels of background respiration were subtracted and mass-specific $\dot{M}o_2$ stated as g O_2 g^{-1} BW h^{-1}. To explore normality and homogeneity of data, residual plots were used. Outliers were identified using boxplots. Mass-independent data of $\dot{M}o_2$ were expressed as residual for standard, routine active metabolic rates and aerobic scope (rSMR, rRMR, rAMR and rAS, respectively) and calculated from least-square linear regression for $\dot{M}o2$ versus body mass as described by Metcalfe et al. (1995). In this study, unlogged plots were used as they provided the best fits for the data and it is appropriate when using a small mass range of individuals (Metcalfe et al. (1995). This method was used in order to identify whether a lobster has a relatively high or low respiration rate for its size and was determined by subtracting the observed $\dot{M}o_2$ for an individual from that predicted for the lobster by the regression between $\dot{M}o_2$ and mass for the population. Positive values indicated an animal with higher than expected $\dot{M}o_2$ for its size, while a negative value indicated a relatively low $\dot{M}o2$.

3.3.5 Data analysis

To test whether the 3-day observations have any effect over the lobsters feeding behavioural outcome, data from 150 feeding contests were analysed using analysis of frequencies, IBM SPSS Statistics version 22.0.

To assess the factors that affect the spiny lobster juveniles dominance status in feeding contest between pairs, an ordinal regression mixed model (ORMM) was fitted with the contest outcome (subordinate, neutral and dominant) as the response variable. Predictor variables for this study were metabolic rate (a continuous variable of individual lobster metabolic status;

rSMR, rRMR, rAMR and rAS), size (a continuous variable of individual lobster carapace length (CL) and carapace length difference (CLD) between pairs), sex (a categorical variable with two levels; male or female), feeding contest experience (a continuous variable of the number of feeding contests conducted on individual lobster) and rearing condition prior to feeding contest (a categorical variable with two levels; individual or communal rearing). The data of the individual lobster's metabolic rate were obtained from the experiment described in Chapter 2 as we predicted that the metabolic rate of the lobsters would be consistent within individuals over the period of time (Alcaraz & Kruesi, 2012 ; Huuskonen et al., 2014)

To analyse the contest outcome, the model used in my study was a cumulative link model for an ordinal response variable Y_i, which fall in $j = 1, \ldots, J$ categories (i.e., subordinated, neutral and dominant). The probability that the ith observation falls in response category j, using logit link, was:

$$logit(\gamma_{ij}) = logit[P(Y_i \leq j)] = log\left(\frac{P(Y_i \leq j)}{1 - P(Y_i \leq j)}\right)$$

With $j = 1, \ldots, J\text{-}1$

And the model that explains variations of that probability given a set of explanatory variables was:

$$logit(\gamma_{ij}) = \theta_j - \beta X$$

With $j = 1, \ldots J\text{-}1$, θ_j is the intercept for each cumulative logit, β is a vector of coefficients and X is a matrix of predictor variables. The factor pairs of lobsters were accounted for as a random factor, as the behaviour in every paired contest was measured over time. The R package *Ordinal* (Christensen, 2015) was used to fit the ordinal model.

To investigate variation in the probability of dominance status based on lobster size, ORMM was conducted using absolute carapace length (ACL) and carapace length difference (CLD) separately along with the other predictor variables. Carapace length was used as the measurement of size as it can be determined more accurately compared to body weight which fluctuates with individual physiological state. Four types of metabolic rate (rSMR, rRMR, rAMR and rAS) were also used to test the effects of different types of metabolic rates on the probability of the spiny lobster's dominance status. Each of the contest outcomes from the pairing tests was treated as an individual independent dominance score (n=100). Model selection criteria were based on the Akaike's Information Criterion (AIC) values which is modelled with the lowest AIC score (Snipes & Taylor, 2014). Only first order interactions were tested to keep parsimony and because higher order interactions are difficult to interpret. The level of significance for all analyses was determined at $P<0.05$.

3.4 RESULTS

3.4.1 Feeding contest behaviour

A total of 150 feeding contests were staged over 3-day observations for each contest pair (n= 50 contest pairs between 33 individuals).

Dominance status could not be determined conclusively in 16 feeding contests (32 observations) because the food was ingested and shared (S) between the pairs and the dominance score of the paired lobsters were categorized as neutral (Figure. 3.3). In the other 134 feeding contests, the dominance scores of the paired lobsters were identified as dominant (FW and C2) and subordinate (NR, O, C1 and FL) individual. The 3-day period of observation showed no significant difference effects on lobsters feeding behaviour outcome (Pearson Chi-Square Test, df= 12, P=0.999) and dominance score (Pearson Chi-Square Test, df= 4, P=0.991).

Subordinates displayed four types of feeding behaviour (NR, O, C1 and FL) and 43.7% of the subordinates behaved non-aggressively (NR, O and C1). The most aggressive behaviour exhibited by the remaining subordinates (56.3%) was displaying attack and retreat behaviour (FL) where the aggressive subordinates launched attacks with their opponents before retreated and lost the feeding contest.

The dominants, conversely, behaved aggressively upon introduction of food. All dominants launched attack behaviour directly with 54.1% taking the food (FW) and 45.9% being the primary consumer by dominating all or most of the food (C2).

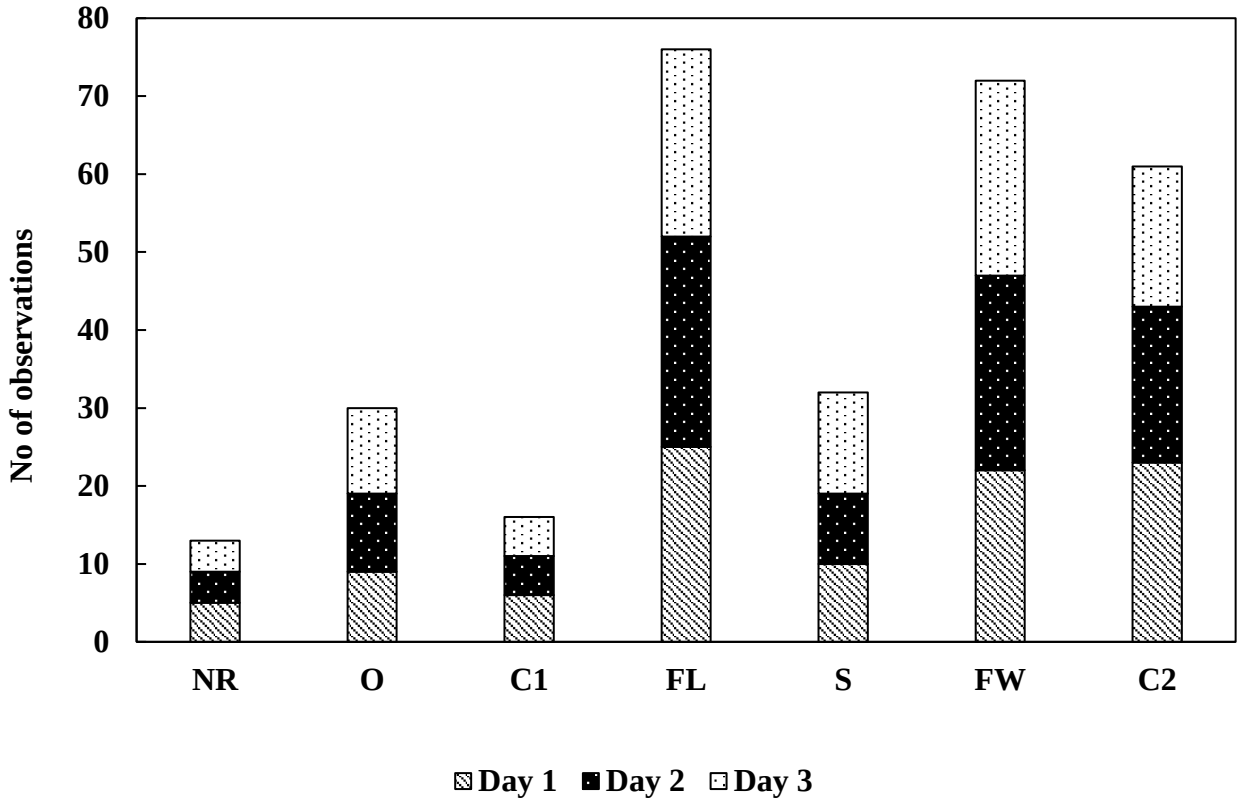

Figure 3.3 Occurrence of feeding behaviour shown by 50 paired lobsters on each of the 3-days pairing contests. NR: no response; O: orientation; C1: secondary consumer; FL: fight and lost; S: share; FW: fight and win; C2: primary consumer.

3.4.2 Effect of size, metabolic rate, sex, feeding contest experience and rearing condition on lobsters' dominance status

Eight models were developed to test the effects of metabolic rate, size, sex, feeding contest experience and rearing condition prior to feeding contest on the probability of a lobster becoming dominant, subordinate or neutral (Table 3.3). The results for the first order interactions given in Table 3.4 indicate that the effect of size (ACL and CLD) was significant (P<0.05) in all the models and indicated that larger lobsters were more likely to become dominant. Model 1 received the lowest AIC score indicating that this model is the most parsimonious model for the given data. Models 2 and 6 demonstrated that the effect of size (ACL and CLD) and rSMR were significant in predicting the lobster's dominance status (P<0.05). Moreover, these models also demonstrated the interaction between size (ACL and CLD) and rSMR indicating that larger individual lobsters with lower metabolic rate were more likely to become dominant (Figure 3.4). Models 1 and 5 demonstrated the effect of size (ACL and CLD), rRMR and sex were significant (P<0.05) predictors of the lobster's dominance status. In addition, size showed an interaction with rRMR and sex. These models revealed that female lobsters have the potential to become more dominant than male lobsters irrespective of their size and metabolic phenotype status (Figure 3.5). Model 3 demonstrated that size (CLD) and sex were significant (P<0.05) on the lobster's dominance status. Furthermore, size displayed an interaction with sex revealing that female lobsters have the potential to become more dominant than male lobsters irrespective of their size (Figure 3.6). In Model 4, 7 and 8, these models demonstrated that only size (ACL and CLD) was significant (P<0.05) on the lobster's dominance status indicating that larger size lobsters have the potential to become more dominant than smaller size lobsters (Figure 3.7 and Figure 3.8). There were no significant (P>0.05) effects of feeding contest experience and rearing condition prior to feeding contest on the lobster's dominance status in all the models.

Table 3.3 Models developed to test the effects of size, metabolic rate, sex, contest experience and rearing condition on dominance status of *Sagmariasus verreauxi* using ordinal regression mixed model (ORMM).

Model	Parameter
1	Size difference + rRMR+ Sex + Experience + Rearing condition
2	Size difference + rSMR+ Sex + Experience + Rearing condition
3	Size difference + rAMR+ Sex + Experience + Rearing condition
4	Size difference + rAS+ Sex + Experience + Rearing condition
5	Absolute CL + rRMR+ Sex + Experience + Rearing condition
6	Absolute CL + rSMR+ Sex + Experience + Rearing condition
7	Absolute CL + rAMR + Sex + Experience + Rearing condition
8	Absolute CL + rAS + Sex + Experience + Rearing condition

CL: carapace length, rSMR: residual standard metabolic rate, rRMR: residual routine metabolic rate, rAMR: residual active metabolic rate, rAS: residual aerobic scope.

Table 3.4**.** Parameter estimates from the ordinal regression mixed model (ORMM) of the effects of lobster's size, metabolic rate, sex, contest experience and rearing condition on the probability of *Sagmariasus verreauxi* dominance status.

Model parameter	**Est**	**S.E**	**Z**	***P***
Model 1: df=8, AIC= 530.3698				
Size: Size difference	0.107	0.018	6.102	1.05e-09*
Metabolic rate: rRMR	-1.834	0.791	-2.318	0.020*
Sex	0.581	0.249	2.336	0.020*
Experience	-0.087	0.097	-0.889	0.374
Rearing condition	0.001	0.285	0.004	0.996
Model 2: df=8, AIC= 531.4828				
Size: Size difference	0.109	0.018	6.219	5.01e-10*
Metabolic rate: rSMR	-2.051	1.000	-2.058	0.040*
Sex	0.469	0.250	1.873	0.061
Experience	-0.086	0.097	-0.886	0.376
Rearing condition	0.017	0.285	0.061	0.952
Model 3: df=8, AIC= 534.3530				
Size: Size difference	0.116	0.018	6.401	1.54e-10*
Metabolic rate: rAMR	-0.670	0.539	-1.241	0.215
Sex	0.574	0.247	2.318	0.020 *
Experience	-0.103	0.098	-1.055	0.291
Rearing condition	0.132	0.275	0.481	0.630
Model 4: df=8, AIC= 534.4311				
Size: Size difference	0.115	0.018	6.414	1.41e-10 *
Metabolic rate: rAS	0.708	0.590	1.200	0.230
Sex	0.474	0.255	1.855	0.064
Experience	-0.102	0.097	-1.047	0.295
Rearing condition	0.148	0.274	0.541	0.589
Model 5: df=8, AIC= 555.7500				
Size: Absolute CL	0.095	0.023	4.142	3.44e-5*
Metabolic rate: rRMR	-2.014	0.762	-2.644	0.008*
Sex	0.481	0.241	1.997	0.046*
Experience	-0.040	0.095	-0.423	0.673
Rearing condition	-0.011	0.280	-0.039	0.969
Model 6: df=8, AIC= 557.8403				
Size: Absolute CL	0.098	0.023	4.254	2.1e-05*
Metabolic rate: rSMR	-2.106	0.947	-2.222	0.026*
Sex	0.363	0.242	1.502	0.133
Experience	-0.039	0.095	-0.417	0.677
Rearing condition	0.012	0.280	0.043	0.966
Model 7: df=8, AIC= 561.2523				
Size: Absolute CL	0.112	0.025	4.590	4.42e-06 *
Metabolic rate: rAMR	0.722	0.546	-1.324	0.186
Sex	0.458	0.238	1.926	0.054
Experience	-0.053	0.095	-0.560	0.575
Rearing condition	0.153	0.269	0.570	0.569
Model 8 4: df=8, AIC= 562.3891				
Size: Absolute CL	0.106	0.024	4.488	7.2e-06 **
Metabolic rate: rAS	0.439	0.568	0.773	0.439
Sex	0.402	0.247	1.629	0.103
Experience	-0.049	0.095	-0.522	0.602
Rearing condition	0.171	0.268	0.638	0.524

* Indicates significant difference (P<0.05).

CL: carapace length, rSMR: residual standard metabolic rate, rRMR: residual routine metabolic rate, rAMR: residual active metabolic rate, rAS: residual aerobic scope.

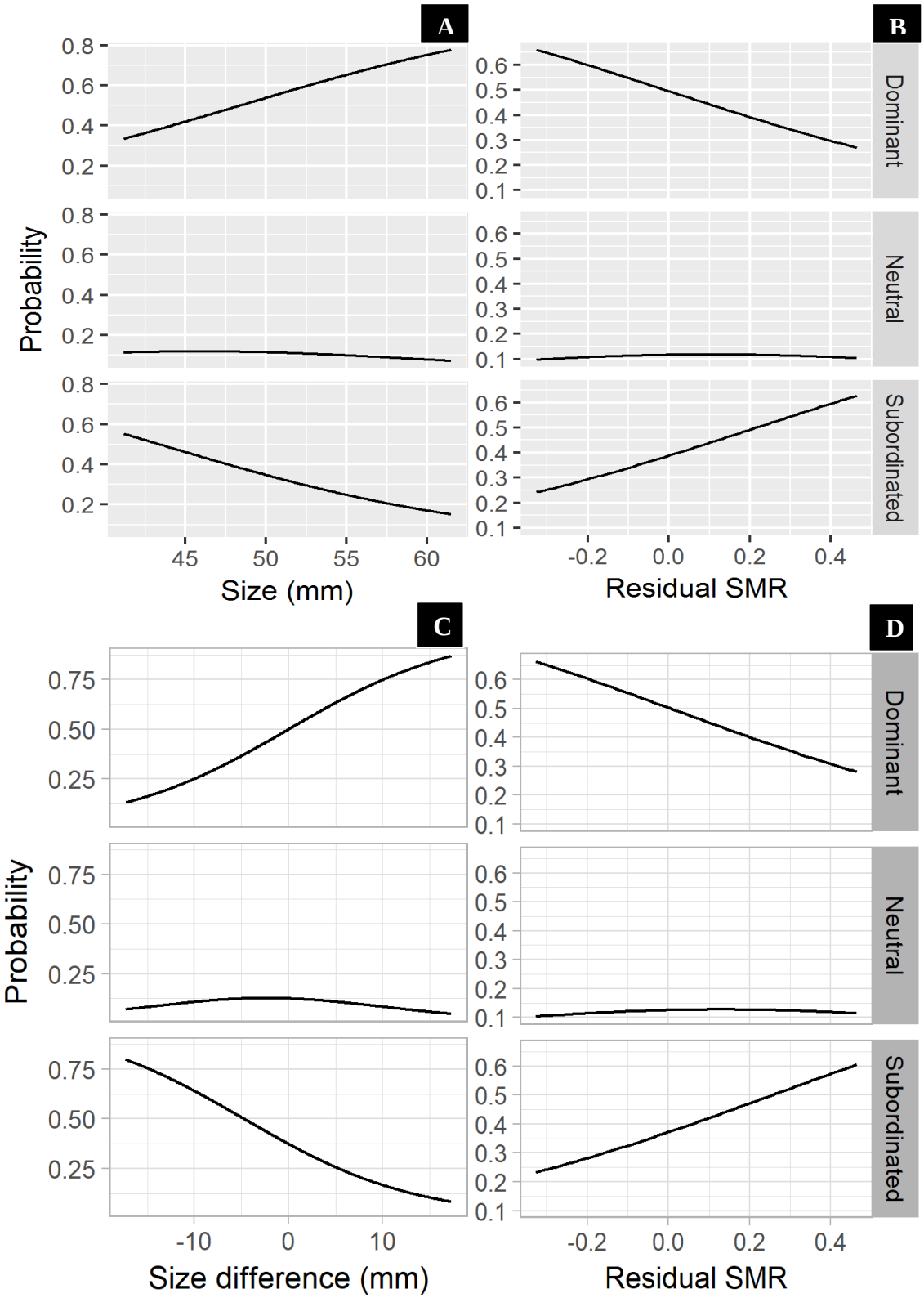

Figure 3.4 Ordinal regression mixed model (ORMM) showing the effect of absolute carapace, carapace length difference and residual standard metabolic rate (SMR) on the probability of *Sagmariasus verreauxi* juvenile lobsters' dominance status. Probability of individual lobsters' dominance status from fitted values Model 2 (C-D) and Model 6 (A-B).

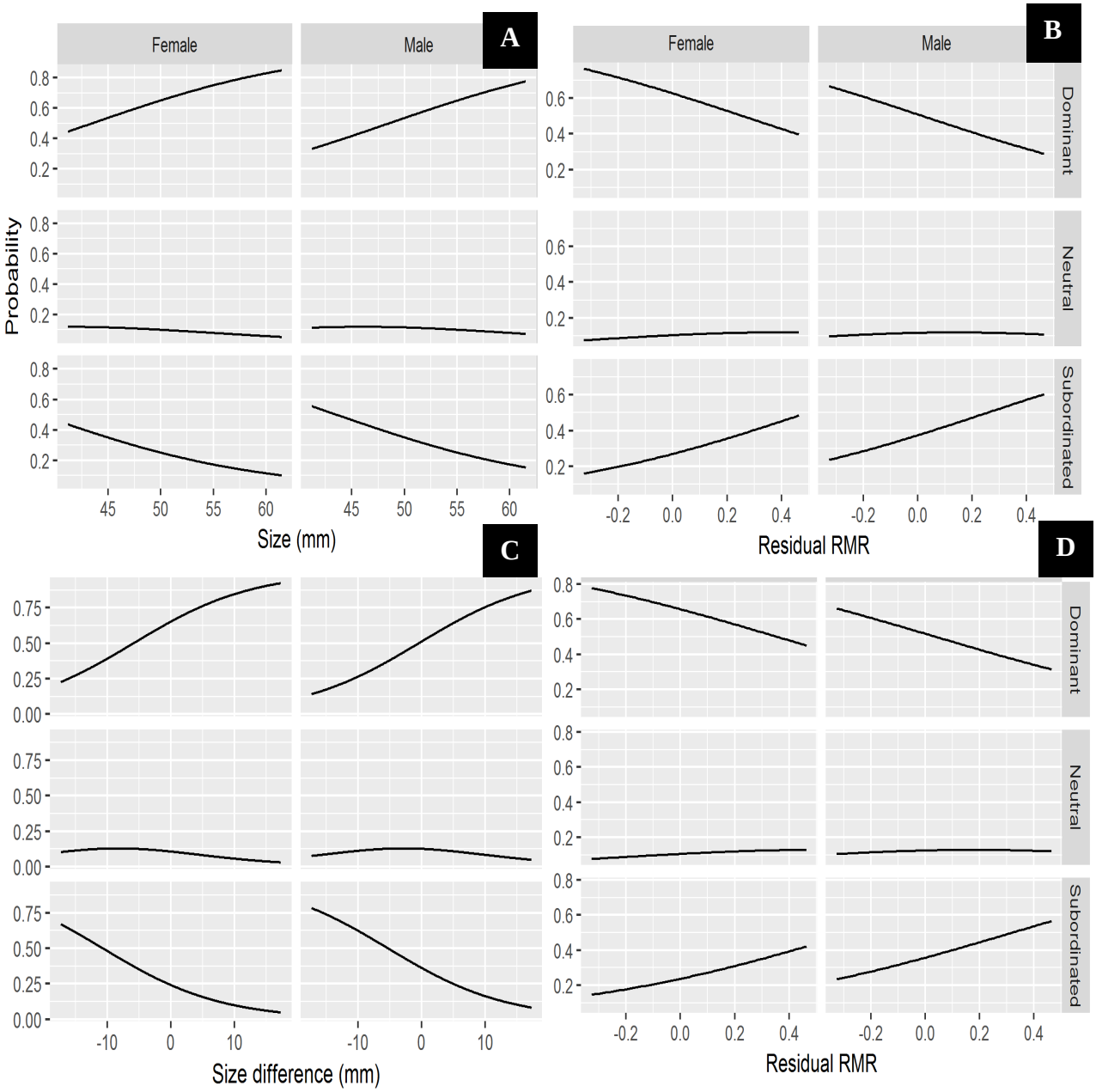

Figure 3.5 Ordinal regression mixed model (ORMM) showing the effect of absolute carapace length, carapace length difference, and residual routine metabolic rate (RMR) on the probability of *Sagmariasus verreauxi* juvenile lobsters' dominance status. Probability of individual lobsters' dominance status from fitted values Model 1 (C-D) and Model 5 (A-B).

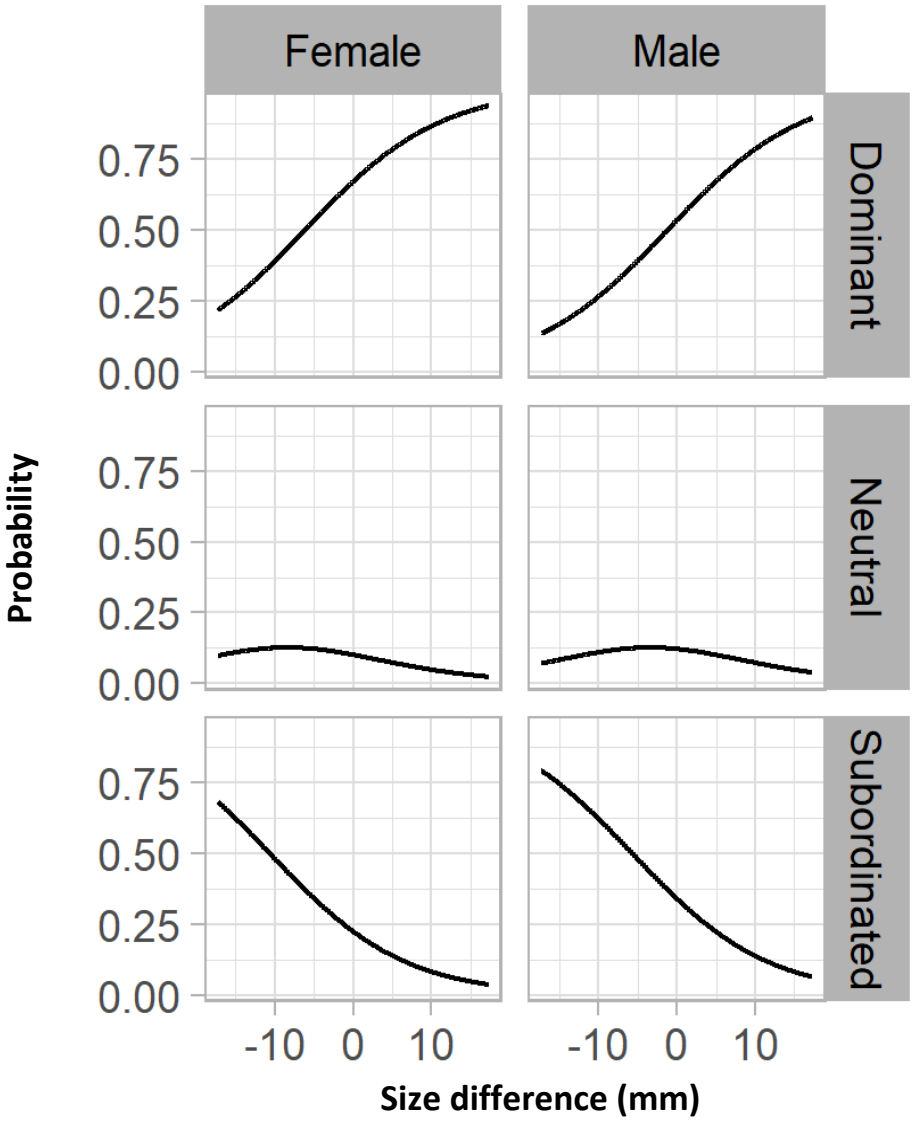

Figure 3.6 Ordinal regression mixed model (ORMM) showing the effect of carapace length difference and sex on the probability of *Sagmariasus verreauxi* juvenile lobsters' dominance status. Probability of individual lobsters' dominance status from fitted values Model 3.

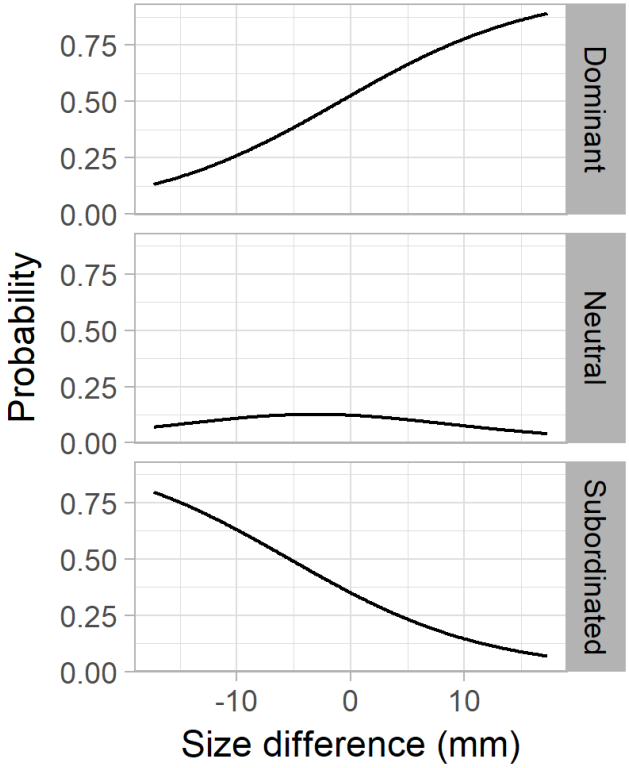

Figure 3.7 Ordinal regression mixed model (ORMM) showing the effect of carapace length difference and sex on the probability of *Sagmariasus verreauxi* juvenile lobsters' dominance status. Probability of individual lobsters' dominance status from fitted values Model 4.

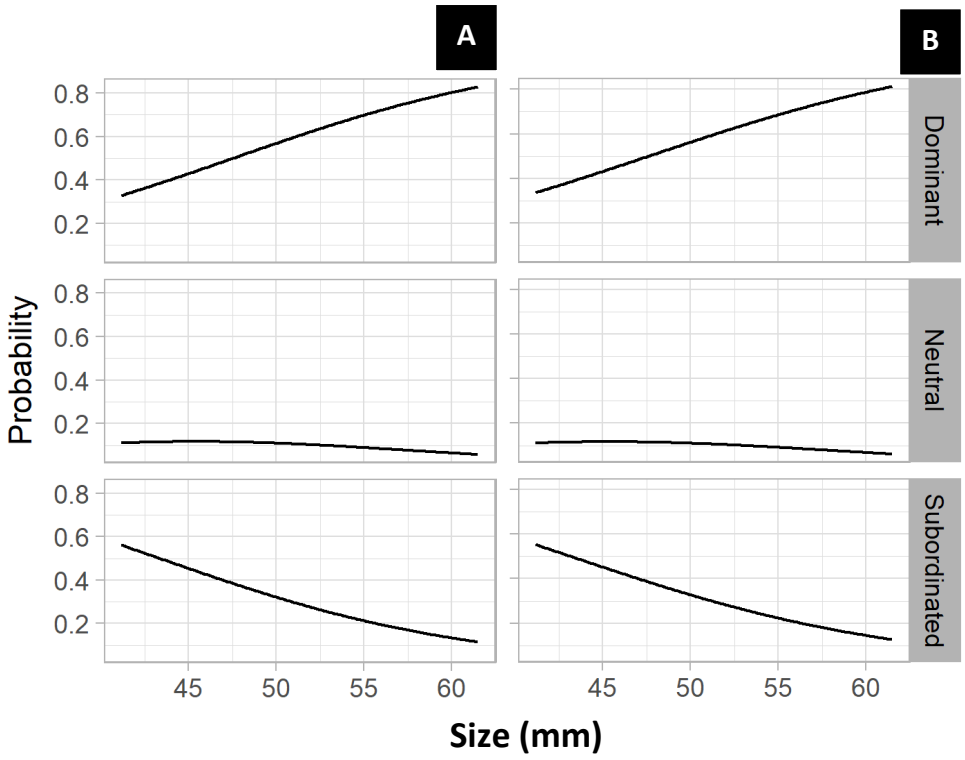

Figure 3.8 Ordinal regression mixed model (ORMM) showing the effect of absolute carapace length on the probability of *Sagmariasus verreauxi* juvenile lobsters' dominance status. Probability of individual lobsters' dominance status from fitted values Model 7 (A) and Model 8 (B).

3.5 DISCUSSION

To the best of my knowledge, no study has addressed the association between the combination of size, metabolic rate, sex, feeding contest experience and rearing history in the feeding-related behaviour and social dominance status of a spiny lobster species. The current findings further demonstrated that lobsters exhibit agonistic behaviour which involves attacking and avoidance-orientated behaviours during feeding contests (Carter et al., 2014). Social dominance status was significantly associated with lobster size, metabolism status and sex. Larger lobsters were predicted to be more dominant over smaller lobsters. At lower metabolic rate, lobsters displayed a greater ability to win over higher metabolic rate lobsters. Moreover, female lobsters showed potential to become more dominant than male lobsters irrespective of size and metabolic phenotype status. This suggests that combination of individual lobster body size, metabolic phenotype and sex can be used as consistent predictors to determine dominance status of spiny lobsters.

3.5.1 Feeding contest behaviour

Agonistic behaviour is a social behaviour involving threats, aggression and submission between individuals that share access to resources, mainly food and shelter and, that may cause direct and indirect effects, such as damage, mortality and growth (Huber & Kravitz, 1995 ; Drengstig & Bergheim, 2013). Previous studies demonstrated that spiny lobsters showed agonistic interactions; mainly during maintenance of social hierarchies such as shelter and feeding hierarchies (Thomas et al., 2003 ; Segura-García et al., 2004 ; Carter et al., 2014). In general, aggressive encounter amongst spiny lobsters also is initiated by the approach of one animal, usually more dominant, towards the other (Cobb & Phillips, 1980). Results from the present study demonstrated that feeding-related behaviours of *S. verreauxi* juveniles involve attacking and avoidance-orientated behaviour which has been described by Huber and Kravitz (1995) as agonistic behaviour. Dominant and neutral lobsters exhibited aggressive behaviour upon introduction of food by launching attack behaviour. Moreover, dominant lobsters also spent more time attacking, fighting and feeding. This agonistic behaviour displayed by the dominant lobsters was similar to the behaviour described in other *Palinurid* species such as *Panulirus cygnus*, *Panulirus interruptus* and *J. edwardsii* (Thomas et al., 2003 ; Cobb & Phillips, 1980 ; Carter et al., 2014). Thomas et al. (2003) described that dominant lobsters chased and attacked other lobsters in defence of their territory and feed which is similar in this study.

Subordinate lobsters displayed agonistic behaviours by demonstrating attack and avoidance behaviours, lost the feeding contests to the dominant lobsters or played a role as secondary consumer.

3.5.2 Effect of, size, metabolic rate, sex, contest experience and rearing condition on dominance status

Social dominance status was significantly associated with lobster's size, metabolism status and sex where female lobsters have the potential to become more dominant than male lobster irrespective of their size and metabolic phenotype status. There was a significant positive relationship between lobster's size and dominance status in all the models suggesting that size was an important predictor of spiny lobster social behaviour status where larger size lobsters are more likely to become dominant than smaller size lobsters, with dominance index increasing as size difference increases between pairs. Previous studies on crustaceans have shown that body size is one of the important predictors in determining the outcome of competition (Ranta & Lindström, 1992 ; Smith et al., 1994 ; Peeke et al., 1995 ; Pavey & Fielder, 1996 ; Figler et al., 1999 ; Thomas et al., 2003 ; Lammers et al., 2010 ; Briones-Fourzán et al., 2014) and may serve as a direct indicator of an individual's competitive ability which can affect status within populations (Silva & de Fátima Arruda, 2015). Early work with *Jasus lalandii* adults (70-90 mm CL) by Fielder (1965b) reported that in communal experiments, larger lobsters often dominate smaller ones in contests over shelter. Similarly, studies on red swamp crayfish *Procambarus clarkii* juveniles (23-32mm CL) by Issa et al. (1999) and Figler et al. (1999) demonstrated that in communal rearing trials, larger individuals were more dominant than smaller individuals, and often ranked highest in the group. Likewise, studies on freshwater crayfish, *Cherax cuspidatus* juveniles (7- 26 mm CL) demonstrated that dominant status was determined based on individual animal body size where larger individuals were likely to become more dominant than smaller body size individuals in a pairing contest. Hence, the current results are consistent with the findings from the previous studies where larger animals were predicted to win over smaller one.

Individual variation in metabolic rate has been widely studied in the context of aggressive behaviour and dominance order in fish and some crustaceans (Metcalfe et al., 1995 ; Brown et al., 2003 ; Metcalfe et al., 2016a). However, the relationship between metabolic phenotype and dominance behaviour of spiny lobsters has not been previously investigated. Earlier studies on *Salmo salar* showed that 70% of the higher metabolic rates juveniles were more dominant than lower metabolic rate juveniles (Metcalfe et al., 1995). Likewise, similar

size female of *M. rosenbergii* juveniles also demonstrated that higher metabolic rates juveniles have a greater potential to win the competition and become more dominant than lower metabolic rates juveniles (Brown et al., 2003). Contrarily, the results from the present study demonstrated that lower metabolic rate lobsters have the potential to become more dominant than higher metabolic rate lobster. Contrasting outcomes could be due to the characteristics of social behaviour in spiny lobsters. *Sagmariasus verreauxi* has been described as a highly sociable, less aggressive and gregarious crustacean species (Berrill, 1975 ; Zimmer-Faust & Spanier, 1987 ; Cobb & Phillips, 1980) while *M. rosenbergii* has been reported to be more aggressive, territorial and exhibits aggressive behaviours (Karplus, 2005).

In the experiment (Chapter 2), using the same lobsters as in this present study, results demonstrated that there is a positive correlation between metabolic phenotype and growth of lobsters in the absence of social interaction. However, the effect of social interaction in communal rearing outweighed the direct link between metabolic phenotype and lobster growth. Findings from this present study may provide an explanation of why lower metabolic rate lobsters have a greater growth rate compare to higher metabolic rate when in communal culture. This may be due to the greater dominance status of lower metabolic rate lobsters allowing improved access to resources such as food. Thomas et al. (2003) reported dominant lobsters have greater ability to control a disproportionate share of food resources compared to the subordinates. Additionally, lower metabolic rate lobsters may also have smaller 'metabolic machinery'. Therefore, as a dominant lobster with a low maintenance cost, there could be a growth advantage for the lower metabolic rate lobsters as stated by Burton et al. (2011) in the 'compensation' hypothesis where greater excess of resources can be directed towards growth.

Previous research demonstrated that sex can confer a competitive advantage along with the individual animal body size (Ranta & Lindström, 1992 ; Peeke et al., 1995 ; Pavey & Fielder, 1996 ; Ueno & Nagayama, 2012 ; Beattie et al., 2012). In this present study, findings showed that sex significantly correlates with dominance status with female juveniles of *S. verreauxi* have the potential to become more become dominant than male juveniles during feeding competition regardless of size and metabolic phenotype status. Early studies on aggressive rank in lobsters suggested that lobster males often showed an advantage over females (Roth, 1972 ; O'Neill & Cobb, 1979 ; Karnofsky & Price, 1989 ; Briones-Fourzán et al., 2014). Similarly, in crabs and clawed lobsters, male individuals often become more dominant than females (Beattie et al., 2012 ; Cobb & Phillips, 1980). However, studies on *Pacifastacus leniusculus* demonstrated a contest advantage of smaller females over larger males for shelter defence and that females can dominate males in social dominance hierarchies

during both the adult and juvenile stages (Momot & Leering, 1986 ; Peeke et al., 1995). On the other hand, Figler et al. (1999) demonstrated a lack of significant advantage of sex in sex-related shelter competition in juvenile *P. clarkii*. This is in contrast to their subsequent findings of a dominance advantage of conspecific adult males over females (Figler et al., 1995a) and findings that females are more aggressive and dominant against conspecific males when ovigerous and brooding but not when non-reproductive (Figler et al., 1995b). Difference in dominance status between males and females among crustaceans may be due to their morphological and physiological variances, which will likely influence the outcome of interference interaction between pairs. For example, in crabs and clawed lobsters, male individuals often have larger chelae than females of equivalent body size which may provide a competitive advantage. Similarly, male spiny lobsters have the first three pair of pereopods, which play an important role in agonistic and mating behaviour, longer and thicker than females (McKoy, 1979 ; Lipcius et al., 1983). This may provide a competitive advantage over females or smaller males or males lacking limbs as reported by (Briones-Fourzán et al., 2014). However, this morphological differentiation does not manifest until sexual maturity and thus was not a factor in the present study with juvenile *S. verrauxii*. Possibly the relationship between sex and dominance status in spiny lobsters may change once lobsters reach sexual maturity which requires further research with *S. verreauxi*.

Contest experience and rearing conditions were not reliable predictors of dominance status in this study as both of these predictors showed no significant effects on contest outcome. According to Karavanich and Atema (1998), *Homarus americanus* recognition of opponents were based on individual's characteristic rather than dominance status where the lobsters were able to differentiate between a familiar and unfamiliar winner. Furthermore, all lobsters that regularly mixed in the communal tank were expected to be able to remember the particular animal that had defeated them in staged encounters. However, in this present study, social interactions during communal culture prior to feeding contest did not provide a detectable advantage for the contest outcome. Previous research by Hoffman et al. (1975) indicates that aggression increases in isolation. Conversely, the results in this study showed being reared individually prior to feeding contest did not affect the contest outcome.

3.5.3 CONCLUSION

The present study is the first to demonstrate evidence that dominance status was related to the size, metabolic rate and sex of juvenile spiny lobsters. Size is an important predictor of dominant status of spiny lobsters with a larger lobster predicted to be dominant over smaller one. Moreover, juvenile females also have a higher probability than juvenile males of becoming dominant at the same size and metabolic rate status. Integration of size, metabolic phenotype and sex can be used as reliable predicators for dominance status and assist in determining growth in captivity. Better understanding of the individual lobster's specific morphological and physiological traits can assist in improving husbandry management strategies which may include monosex culture and size grading.

CHAPTER 4

GROWTH VARIATION IN SOUTHERN ROCK LOBSTER, *JASUS EDWARDSII*: THE INFLUENCE OF EMERGENT JUVENILE BODY SIZE, METABOLIC PHENOTYPE AND SOCIAL BEHAVIOUR

4.1 ABSTRACT

Southern rock lobster, *Jasus edwardsii* possibly has among the most extended larval phase known for any marine invertebrate, developing through 11 moult stages. After metamorphosis, the emergent juvenile can display high variance in body size which could be linked to their larval experiences. The larval experience and size and condition of post-metamorphosis individuals are known to be the important factors affecting the individual variation on growth performance of juvenile aquatic invertebrates. However, the effect of emergent juvenile size on subsequent growth performance of individual spiny lobsters has not been investigated. In a laboratory experiment, the influence of body size, metabolic phenotype and social behaviour on growth increments of *J. edwardsii* emergent juveniles were examined. The growth of sixty post-pueruli (10.98±0.71 mm carapace length) that were reared either individually (n=30) or in a group of ten communally (n=3) for over four moult cycles was determined. Lobsters reared communally had a larger carapace length and took less time to complete the first four moult cycles than those reared individually. This suggested aspect of social interaction increased the lobster's growth performance. Body size of the emergent juveniles had no significant effects on growth increments in either rearing treatment. Lobster growth in the individual rearing treatment was positively correlated with routine metabolic rate, active metabolic rate and aerobic scope, while the communally reared lobsters showed no correlation suggesting that metabolic phenotype is an important determinant of growth in the absence of social interaction. The results of this study displayed that growth performance of spiny lobsters in correlated with individual metabolic phenotype with social behaviour playing an important role in determining the growth of individual lobsters.

Keywords: growth, body size, metabolic phenotype, social behaviour, individual variation

4.2 INTRODUCTION

The southern rock lobster, *Jasus edwardsii,* is a temperate species of spiny lobster which is widely distributed throughout coastal waters of southern Australia and New Zealand (Jeff et al., 2001a ; Jeffs et al., 2013). This species represent as one of the most important fishing resources in Australia and New Zealand and has also been identified as a potential aquaculture species (Crear et al., 2000 ; Jeffs, 2010). Similar to most spiny lobsters under aquaculture conditions, *J. edwardsii* displayed size disparity among individuals from the same cohort due to the difference in individual growth performance (Crear et al., 2000). To date, research has investigated the factors that may influence spiny lobster growth performance in captivity (Thomas et al., 2000 ; Crear et al., 2000 ; Thomas et al., 2003 ; Carter et al., 2014). However, the mechanisms underlying how inter-individual variation in body size can influence individual lobster's growth performance have been largely overlooked.

Like other spiny lobsters, *J. edwardsii* demonstrates a long larval life phase, comprising with free swimming planktonic larvae, phyllosoma, that spend between 8-24 months in oceanic water before they metamorphose into the final larval stage the puerulus (Ventura et al., 2017), which migrate back to shore to find suitable benthic settlement substrate (Edmunds, 1995). The puerulus is a non-feeding stage that supports energy demands with stored reserves accumulated during the preceding phyllosoma phase (Fitzgibbon et al., 2014). Following settlement, the puerulus completes metamorphosis into the emergent juveniles stage (Ventura et al., 2015; Ventura et al., 2017). Post- metamorphosis, emergent juveniles of *J. edwardsii* can display high variance in body size with post-puerulus (first instar juvenile, J1) ranging from 10-15 mm carapace length (CL) (MacDiarmid, 2011). This size disparity of the emergent juveniles may result from differences in environmental and nutritional condition experienced by individuals during the larval development phase and could correspond to inter-individual variation in condition of emergent juveniles (Jeffs et al., 2001a ; Jeffs et al., 2001b). It has now been recognized for many years that within-species variation in juvenile performance of aquatic invertebrates can reflect differences in embryonic or larval experiences (Pechenik et al., 1998; Phillips, 2002; Pechenik et al., 2002; Marshall et al., 2003; Phillips, 2004; Emlet & Sadro, 2006; Pechenik, 2006; Marshall & Morgan, 2011). These so called "latent effects' have their origins in early development but manifest during juvenile or adult development (Phillips, 2002; Phillips, 2004; De Block & Stoks, 2005; Emlet & Sadro, 2006; Giménez, 2006; Pechenik, 2006). Latent effects have been shown to contribute to individual variation of a wide range of performance parameters including growth rates, survival, competitive ability and environmental tolerances in numerous aquatic species (Marshall & Keough, 2006; Giménez,

2010; Crean et al., 2011; Diederich et al., 2011), and has led to the phrase that "metamorphosis is not a new beginning (Pechenik, 2006). Considering the extended duration and complexity of spiny lobster larval development, it might be expected that latent effects would be profound for the species. However, until now, no study has been carried out to investigate how inter-individual body size of the emergent juveniles may influence the growth performance of the emergent spiny lobster juvenile.

Information on the effects of metabolic phenotype on individual animal growth performance is important to understand the fitness of the organism. Metabolic rate is an important physiological trait of an individual as aerobic energetic costs and capacities are fundamental for an organism to perform and function in order to support life (Hulbert & Else, 2000). Metabolic phenotype is also considered central for explaining patterns of adaptation and life history diversity of individual animals within species (Brown et al., 2003 ; Biro & Stamps, 2010 ; Metcalfe, 2015). Studies on fish and crustaceans have reported that inter-individual variation in metabolic phenotype can affect the growth, reproduction, survival, life span and behaviour of individual animals (Metcalfe et al., 1995 ; McCarthy, 2000 ; Brown et al., 2003 ; Perera et al., 2007 ; Metcalfe et al., 2016). It is also well established that metabolic phenotype can vary greatly among individuals within populations which can correspond to variation in food consumption, growth and behaviour (Burton et al., 2011 ; Auer et al., 2015 ; Metcalfe et al., 2016 ; Killen et al., 2017). Likewise, in Chapter 2 we demonstrated the direct link between metabolic phenotype and lobster's growth performance by *Sagmariasus verreauxi* juveniles.

Behaviours such as dominance and aggression are also reported to be important in spiny lobster aquaculture (Briones-Fourzán et al., 2008 ; Briones-Fourzán et al., 2014 ; Carter et al., 2014). Individual variation in behaviour of aquatic organisms can be influenced by metabolic phenotype (Reid et al., 2011 ; Reid et al., 2012). Studies on teleosts have shown that higher metabolic rate individuals can be more dominant, aggressive and grow faster resulting in a size advantage that ensures a higher rank in a social hierarchy (Metcalfe et al., 1995 ; Biro & Stamps, 2010 ; Metcalfe et al., 2016). Similarly, studies on crustaceans have also shown that metabolic phenotype can be important in social interaction which may affect their growth and survival in rearing environments (Smith & Taylor, 1993 ; Thorpe et al., 1995 ; Taylor et al., 2002 ; Brown et al., 2003). Early work has demonstrated that gregarious animal species are often divided into distinct social groups according to various morphological phenotypic traits, including size, age, and sex (Krause & Ruxton, 2002 ; Hoare et al., 2004). Moreover, gregarious animals often display wider behavioural differences among individuals within a cohort with some individuals being consistently more active, aggressive, and dominant and

more exploratory (Yamamoto et al., 1998 ; Brown et al., 2003 ; Killen et al., 2013). This variation in behaviour is thought to be associated with an individual animal's physiological traits together with other morphological traits, as demonstrated for *S. verreauxi* juveniles in Chapter 3, where individual lobster's dominance status was determined by their body size, sex and metabolic phenotype.

Understanding how variation in body size at metamorphosis, metabolic phenotype and social behaviour can influence the growth performance of spiny lobsters, particularly in captivity, is important because the information is necessary for the establishment of optimum rearing conditions and management of spiny lobster culture. The present study describes the growth (intermoult duration, moult increment, and moulting duration) of post-puerulus *J. edwardsii* in relation to body size at post-metamorphosis, metabolic phenotype and social behaviour. To test the influence of social behaviour of post-pueruli the experimental trials were conducted both in communal and individual rearing conditions.

4.3 MATERIALS AND METHODS

4.3.1 Experimental animals

Wild pueruli of *J. edwardsii* were collected between October to December 2016, using crevice collectors similar in design to those described by Booth and Tarring (1986). Collectors were deployed at three coastal locations in Tasmania, Australia; Bicheno (41.8713° S, 148.3024° E), Recherche Bay (43.5938° S, 146.9187° E) and South Arm (43.0530°S, 147.4169°E) Tasmania (Figure 4.1). A mesh bag was placed around the collector by a diver before the collector was retrieved to the surface and pueruli collected. Live pueruli were transported to the Institute of Marine and Antarctic Studies, Taroona on the same day of collection and communally reared in a plastic vessel (55cm length X 21 cm width X 12.5cm height) which received flow-through filtered sea water at three exchanges per hour. Pueruli were maintained at 18^{o}C with a light regime of 16 h light and 8 h dark until the pueruli moulted to post-pueruli also known as first instar juvenile (J1) or emergent juvenile. Feeding was not provided during the lecithotrophic puerulus stage.

A sample of 14 emergent juvenile was obtained in October which were stocked into the individual rearing treatment (Table 4.1). A sample of 42 were obtained in November which were split between the communal and individual rearing treatment and a sample of four were obtained in December which were used for individual rearing treatment.

4.3.2 Individual and communal rearing experiment

Growth experiments were conducted to examine the growth (intermoult duration, moult increment, and moulting duration) survival, final body weight, and condition of the emergent juveniles at two different rearing treatments; individual and communal rearing.

Individual rearing treatment consisted of four rectangular plastic vessels (55cm length X 21 cm width X 12.5cm height each of) equally divided into eight compartments (6.8cm length X 21 cm width X 12.5cm height). To eliminate individual lobster chemical interaction, each compartment was isolated and individually supplied with filtered seawater at three exchanges per hour. Thirty lobsters were placed individually into a compartment, with a single 6 cm length cylindrical shelter made from 25mm polyvinyl chloride (PVC) pipe. Each lobster were treated as replicates for the individual rearing treatment (n= 30 individuals).

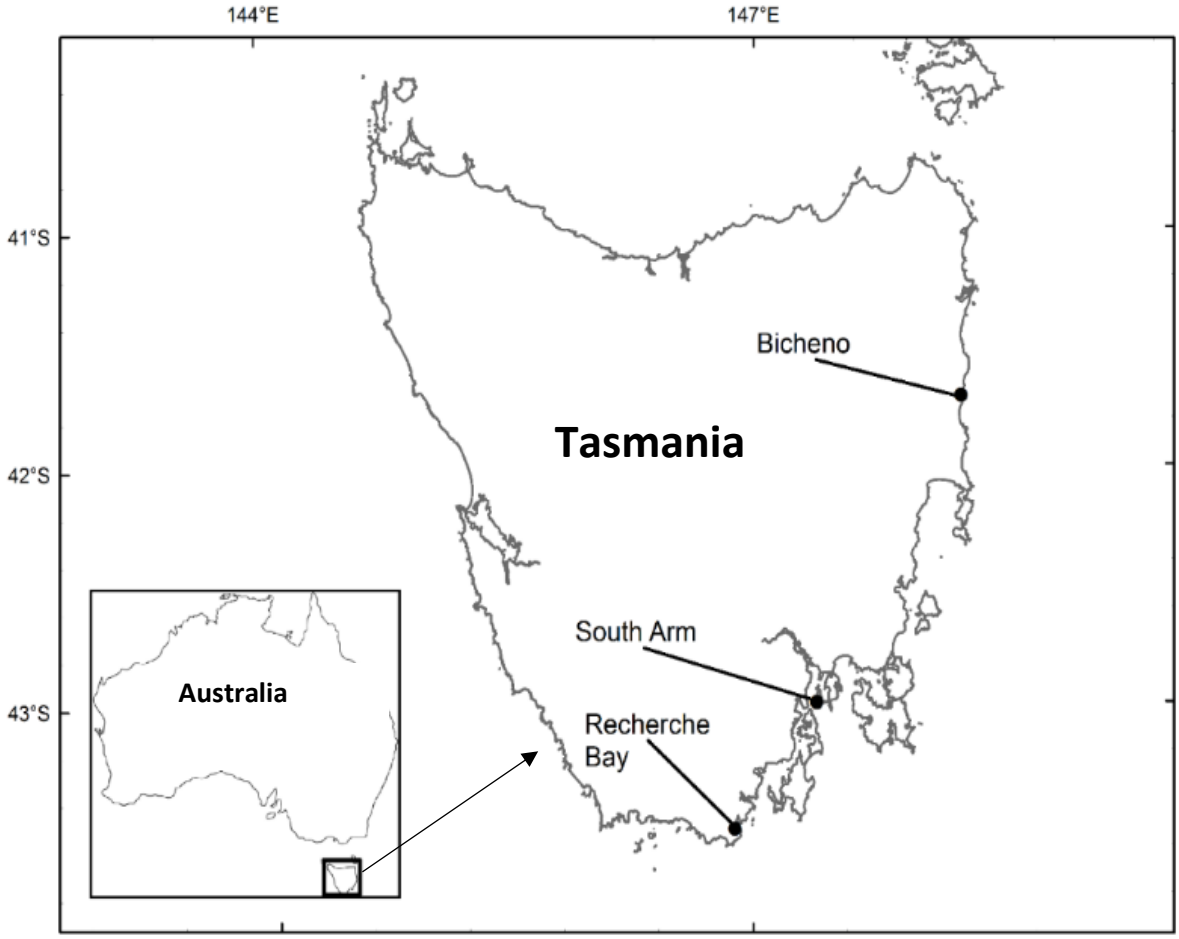

Figure 4.2 Map of Tasmania, Australia, showing the locations for the collection of pueruli used in the study

Table 4.1 Number of *Jasus edwardsii* pueruli collected monthly from four different collection periods.

Collection period	Individual	Communal
End-October 2016	14	0
End-November 2016	12	30
Early-December 2016	1	0
Mid- December 2016	3	0
Total	30	30

The communal rearing treatment consisted of three replicates of 10 lobsters reared in rectangular plastic vessels (40 cm length X 27.5cm width X 25 cm height) filled with 10 l of filtered seawater at three exchanges per hour and 10 single cylindrical shelters as previously described.

The water quality parameters in both rearing treatments (pH, dissolved oxygen and temperature) were measured daily and temperature was maintained at 18^{o}C, salinity 33-35, pH 8.1 and 80-100% oxygen saturation with a 12:12 photoperiod. Lobsters in all treatments were fed to excess once per day with a commercially confidential moist formulated diet and fresh blue mussels (*Mytilus galloprovincialis*) gonad and mantle. All uneaten feed was removed from each treatment immediately before subsequent feeding and the vessels were cleaned daily. The growth experiment was terminated after all the lobsters moulted to fifth instar juvenile stage (J5).

To differentiate each individual lobster, numbered polymer tags were glued (Loctite 454 ®) to the carapace before the post-pueruli were transferred into the rearing container. Visible Implant Elastomer tags (VIE) was also injected on the edge of the 1st abdominal segments three days after moulting (Woods & James, 2003). Moulted lobsters were identified as lobsters without tag and VIE colour, moult date recorded and re-tagged.

Images of each lobster were taken using a digital camera (Canon G12) once the moulted lobsters had hardened sufficiently (approximately 72 hours post moult). Images were used to determine lobster carapace length (CL; mm). Using the captured image, CL was measured along the dorsal line from the rostrum to the dorsal margin of the carapace using an ImageJ (Image Processing and Analysis in Java, https://imagej.nih.gov/ij/) software. Images were calibrated against images with known values (scales) and same magnification, then applying that calibrated image to unknown images.

Lobster growth was measured by quantifying the intermoult period (INT), carapace length increment (ΔCL), and moulting period (day) from J1 to J5 stage (D). Lobster's whole-body wet weight (WW, g) was measured at the termination of the experiment (J5).

The period (days) between consecutive moults was defined as the intermoult period (INT). D was defined as the mean duration of days individual lobsters took to moult from J1 to J5. The percentage moult increment (ΔCL, %) was calculated as the carapace length increase between J1 and final J5 as follows:

$$\Delta \mathbf{CL}\ (\%) = \frac{\mathbf{CL\ J5 - CL\ J1}}{\mathbf{CLJ1}} \boldsymbol{X}\mathbf{100}$$

Dry body weight (DBW) was measured after the oxygen consumption rate measurement. Lobsters were euthanized in an ice bath for 5 min before rinsing with distilled water to remove any salt, stored at -20 and freeze dried to a constant weight at -55ºC.

The coefficient of variation (CV) was measured to examine the variability of DBW (CV_{DBW}), ΔCL ($CV_{\Delta CL}$), CL (CV_{CL}), D (CV_D) and INT (CV_{INT}). CV_{DBW} and $CV_{\Delta CL}$ were examined to determine the influence of rearing treatment on the variability of individual lobsters' size disparity and growth increment (McCarthy et al 1992). CV_{CL} were investigated to determine the variability of individual lobsters CL size in each juvenile stage within rearing treatment. CV_D and CV_{INT} were examined to determine the influence of rearing treatment on moult synchrony. All CVs were calculated as follow:

$$\mathbf{CV}\ (\%) = \frac{\mathbf{S.D}}{\mathbf{mean}}\mathbf{X\ 100}$$

4.3.3 Oxygen consumption rate ($\dot{M}o2$)

The oxygen consumption rate ($\dot{M}o_2$) of 59 J5 lobsters were measured using automated intermittent flow-through respirometry similar to that described by Fitzgibbon et al. (2014) after the completion of the growth experiment. One lobster from the individual rearing treatment died during the $\dot{M}o_2$ measurement and was not considered in the analyses of metabolic rate effects. The respiratory system consisted of four 50 ml polypropylene respiratory chambers (50 ml conical bottom centrifuge tubes) with an internal diameter of 30 mm and a length of 115 mm submerged in a water bath (24cm height x 24 cm length and 25 cm width). The respiratory system received a continuous 18ºC seawater supply at a rate of 17 l h^{-1}. Two twin channel mini peristaltic pumps (Harvard MP II mini peristaltic pump) were used to continuously circulate water at a rate of 10 ml min^{-1} through the chambers and past an

oxygen sensor. Dissolved oxygen was recorded and logged every 20 s by a fibre optic oxygen microsensor meter (OXY-4 mini, www.preSense.de) connected to a computer. To introduce new water from the external water bath into the chamber, another two-twin channel peristaltic pumps were used and water was circulated at a rate of 14 ml min^{-1}. The pump was connected to a digital recycler timer (Sentinel DRT-1) and was programmed to turn on in 10 min and 5 min off cycles which allowed a $\dot{M}O_2$ measurement every 15 min. To maintain the dissolved oxygen concentration in the external water bath at 100% saturation, an aquarium air stone was used to supply air. In order to exclude light and external stimuli, a black corflute screen was used to enclose the water bath housing. Throughout the $\dot{M}O_2$ measurements, dissolved oxygen within the respiratory chambers never fell below 85%. Following each experiment, the respiratory system was sterilized with a 1 mg l^{-1} solution of sodium hypochlorite, rinsed with fresh water and air dried.

Oxygen consumption rate evaluations and metabolic states of all lobsters were evaluated similar to that described by Fitzgibbon et al. (2014). The moulting of all lobsters was monitored during the growth experiment. $\dot{M}O_2$ of individual lobsters was measured after completion of the growth experiment to avoid experimental stress which may have affected the growth results. $\dot{M}O_2$ measurements were taken during the intermoult phase which was defined from day 7 to 15 post moult. Before $\dot{M}O_2$ measurement, the lobsters were starved for 24 hours to clear the digestive tract of food and faeces, and to eliminate variability of measurements associated with thermic effect of food (total energy expenditure above the SMR due to the cost of processing food for use and storage) or also known as specific dynamic action (SDA). In the late evening after 24 h of starvation, individual lobsters were placed into the respirometer chambers and $\dot{M}O_2$ logged overnight for 16 h (approximately 64 $\dot{M}O_2$ measurements). Standard metabolic rate (SMR) was defined from the mean of the lowest five recording of the $\dot{M}O_2$. The average of all the 64 recordings was defined as routine metabolic rate (RMR). To stimulate active metabolic rate (AMR), lobsters were removed from the respirometer chamber and made to swim by encouraging the lobster by hand to swim inside a small tub (40cm height X 60 cm length and 30xm width) until the lobster became exhausted and non- responsive to stimuli (approximately 10 min). Lobsters were then transferred back into the chamber and $\dot{M}O_2$ was recorded for 2 h. Exhaustion protocol was maintained to keep in time with the open cycle of the respirometer system to allow immediate $\dot{M}O_2$ measurements. AMR was defined based on the highest 10% recordings of the $\dot{M}O_2$ measured after the exhaustion exercise. Aerobic scope (AS) was determined by subtracting the SMR from the AMR. As a measurement of background respiration, the oxygen demand of the respirometer system was then recorded for another 2 h.

The lobster $\dot{M}O_2$ were determined using linear regression on the rate of decline of dissolved oxygen concentration over the final 4 min of each 5 min respirometer closed cycle period. When the R^2 of the linear regression was below 0.95, data for the period were excluded from analysis. Lobsters' mass-specific $\dot{M}O_2$ were expressed in mg O_2 h^{-1} g WW^{-1} after the subtraction of mean background respiration. Following measurements of $\dot{M}O_2$, all lobsters were removed from the chambers and the whole-body wet weight (WW, g) was measured after removing excess moisture with paper towel.

4.3.4 Data analysis

To explore homogeneity of variance and normality, data were tested using Levene's test and the Shapiro-Wilk test respectively. Individually housed lobsters were treated as replicates (n= 30) and three replicates of communally housed lobsters (n=3).

One-way ANOVA and Tukey's HSD post hoc test was used to test differences in lobster growth performance reared individually with collection periods (month) as a fixed factor. To test differences in final DBW, ΔCL and D among rearing treatments, one-way ANOVA was used with rearing treatment as a fixed factor. Data for lobsters CL, INT and ΔCL were compared in a three- way ANOVA (rearing treatment X juvenile stage X sex).

Carapace length increment were expressed as residual carapace length (rΔCL). Expected ΔCL was achieved by calculating the linear regression of observed ΔCL on initial CL (CL at J1) and rΔCL were determined from the regression line (the difference between observed and expected ΔCL). Lobsters with higher value of ΔCL than the expected for their size had positive values of rΔCL while those with lower value of ΔCL than expected had a negative rΔCL. Linear regression was performed to examine the effect of rearing treatments on the interaction between moulting duration and rΔCL. To examine the influence of initial size (CL_{J1}) on lobster growth; percentage of ΔCL, final CL (CL_{J5}), DBW and moulting duration (D), linear regression was performed and tested for significance by ANOVA.

To investigate the relationship between mass-specific $\dot{M}O_2$ (mg O_2 h^{-1} g^{-1}) and DBW (g) linear regression analysis were used. Mass-specific $\dot{M}O_2$ data were expressed as residual metabolic rates (rSMR, rRMR, rAMR and rAS) calculated from linear regression of observed $\dot{M}O_2$ on body weight as described by Metcalfe et al. (1995). For this experiment data, unlogged plots were used as it was a better fit and appropriate when using a small mass range of individuals (Metcalfe et al., 1995). Residual (body weight corrected) $\dot{M}O_2$ (r$\dot{M}$o2) were determined from the regression line (the difference between observed $\dot{M}O_2$ and expected $\dot{M}O_2$). The lobsters with higher rates of oxygen consumption than the expected for their size had

positive values of r$\dot{M}O_2$ while those with lower respiration rates than expected had a negative. Relationships were compared between on rΔCL with rSMR, rRMR, rAMR and rAS using linear regression analysis. ANOVA was used to test for significance in all linear regression lines.

All analyses were performed using the IBM SPSS Statistics version 22.0. The level of significance for all analyses were determined at $P<0.05$. Values were presented as mean ± standard error (S.E) unless stated otherwise.

4.4 RESULTS

4.4.1 Size distribution of the emergent juvenile

The carapace length of the emergent juveniles of *J. edwardsii* covered a wide range from 9.24 to 12.79 mm and was normally distributed around an average value of 11.28 mm CL (Figure 4.2). The initial CL of the lobster reared individually and collected in December was significantly larger than those collected in October and November (Table 4.2). However, there was no significant effect of collection period on growth performance of lobster reared individually.

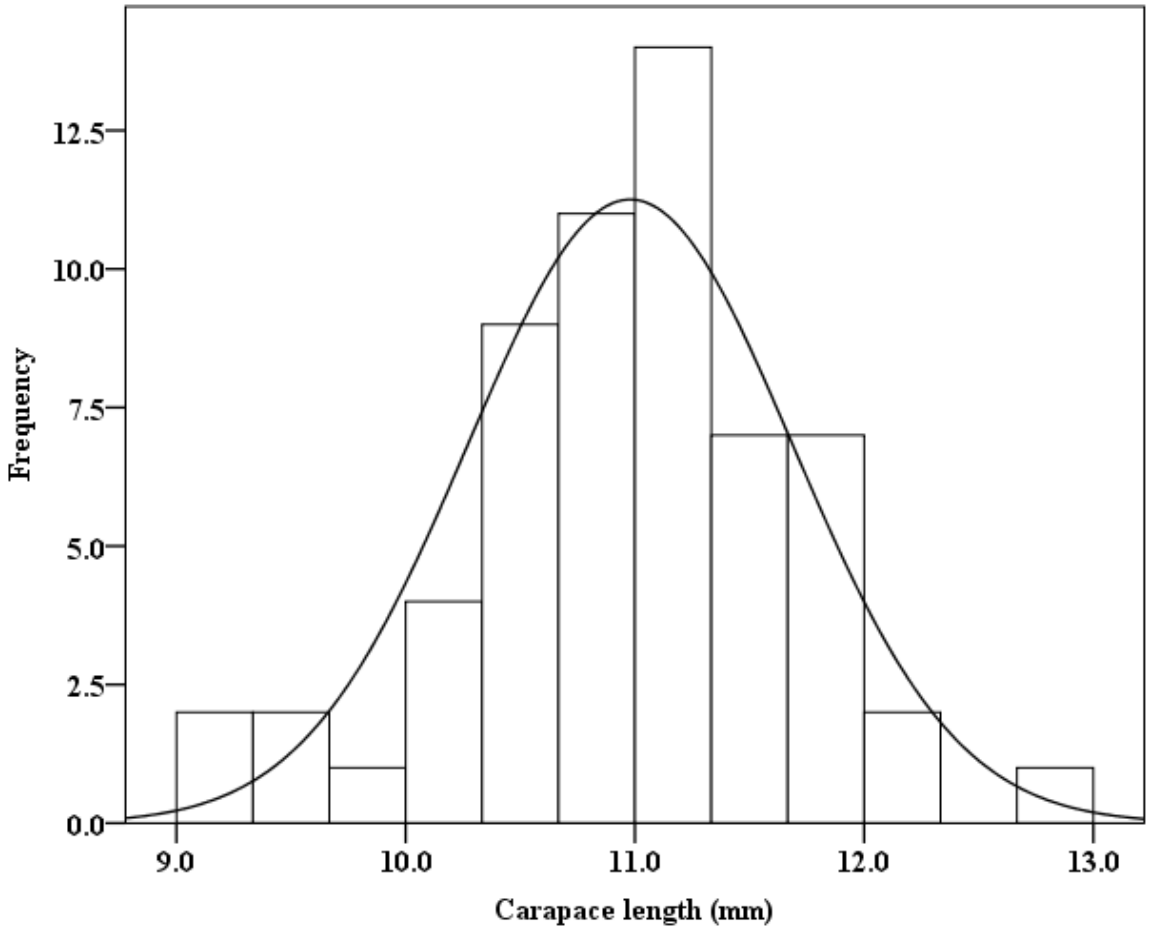

Figure 4.2 Carapace length and size distribution of the emergent juvenile *Jasus edwardsii* at first instar juvenile (J1) stage (n=60)

Table 4.2 Comparison of the growth performance of *Jasus edwardsii* emergent juvenile collected in October (n=14), November (n=12) and December (n=4). All lobsters were reared individually up to J5 stage. Significant differences between means within the same row are maked by different letters in superscript (one-way ANOVA, $P < 0.05$)

Parameters	October	November	December	*F*	*df*	*P*
Initial CL (CLJ1) (mm)	10.70 ± 0.15^{a}	10.70 ± 0.24^{a}	12.01 ± 0.39^{b}	4.456	2, 27	0.021
Final CL (CLJ5) (mm)	22.46±0.56	22.77±0.40	23.66±0.94	0.559	2, 27	0.578
Final DBW(g)	1.30±0.09	1.71±0.05	1.30±0.08	0.230	2, 27	0.796
ΔCL (%)	110.53±6.12	113.97±6.00	97.77±13.22	0.642	2, 27	0.534
D (days)	125.4±8.26	129.0±8.04	125.67±2.40	0.055	2, 27	0.946

CL: carapace length, DBW: dry body weight, ΔCL: carapace length increment, D: intermoult period (days) from J1 to J5 stage, CLJ1: carapace length at 1st instar juvenile, CLJ5: carapace length at 5th instar juvenile.

4.4.2 Effect of emergent juveniles' body size on growth

The relationships between lobster's carapace length (CL_{J1}) and final CL (CL_{J5}), DBW, rΔCL and D are shown in Figure 4.3. Linear regression in Table 4.3 demonstrated that lobster's body size did not significantly ($P > 0.05$) influence these growth parameters.

4.4.3 Effect of social interaction on growth and survival

The initial mean carapace length (CL_{J1}) of the lobster in each rearing treatment was not significantly (Figure 4.4A). There was significant interaction between rearing treatment and juvenile stage on lobster's growth with communally reared lobsters growing faster than those reared individually (Table 4.4). Differences in growth between lobsters in both rearing treatments became significantly apparent over time because of differences in carapace length size, moult increment and intermoult period (Table 4.4, Figure 4.4). Rearing the lobsters communally resulted in significantly greater carapace length and moult increment and decreased the intermoult period. There was no effect of sex on either carapace length, moult increment or intermoult period of the lobsters in both rearing treatments. Lobsters reared communally demonstrated significantly (one-way ANOVA, F= 5.773, df = 1, 58, P= 0.019) greater final dry body weight (1.51±0.06g) and shorter total moulting period (98.63±1.44 days) than those individually reared (1.33±0.05g, 127.0±5.10 days). All lobsters in both treatments survived the growth experimental period. Moulting period throughout the rearing experiment showed no significant relationship with residual carapace length increment (rΔCL) in both rearing treatments ($P > 0.05$) (Table 4.5, Figure 4.5)

Coefficient of variation (CV) of final DBW of communally reared lobsters was 21.38±2.35% which was higher than lobsters reared individually (19.60%) indicating a wider variability in DBW of communally reared lobsters. CV_D of communal rearing (6.97±2.02%) was lower than individual rearing (21.99%) indicating less variability in moulting period of the population in communal rearing. The CV_{CL} for lobsters reared communally increased with the juvenile stage indicating size disparity among communally reared lobsters becoming more apparent as the lobsters grow (Table 4.6). CV_M for individually reared lobsters was greater than communally reared lobsters indicating the moult increment in the individual rearing population has a greater range of variability. The CV_{INT} of communally reared lobsters was lower than individually reared lobsters in each intermoult stage and, decreased from the second intermoult period (INT_2) indicating the moulting patterns of the communally reared population became more synchronous with time.

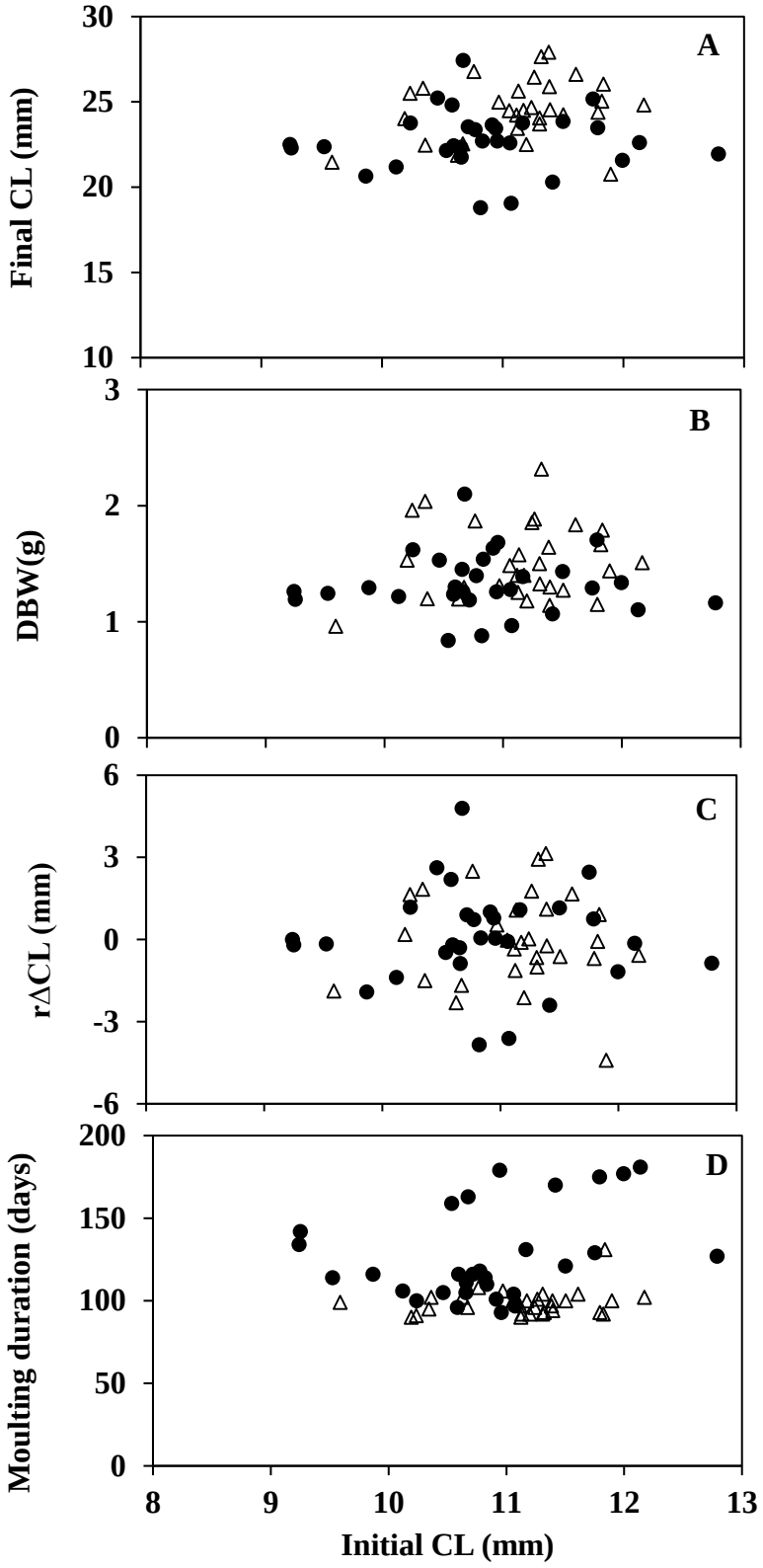

Figure 4.3 Relationship between *Jasus edwardsii* emergent juvenile carapace length (initial CL) and A) final carapace length (Final CL), B) dry body weight (DBW), C) residual carapace length increment (rΔCL), D) moulting period reared individually (●) and communally (Δ). Each data point represents an individual lobster. Details of regression are presented in Table 4.3

Table 4.3 Details of linear regression (y=a+bx) describing the relationship between *Jasus edwardsii* emergent juvenile carapace length with final carapace length (CL), final dry body weight (DBW), residual carapace length increment (rΔCL) and moulting period (D) presented in Figure 4.3 (ANOVA, $P < 0.05$).

	Treatment	A	b	r^2	*P*
Final CL (mm)	Communal	15.801	0.788	0.068	0.163
	Individual	22.084	0.058	0.001	0.890
Final DBW (g)	Communal	0.880	0.057	0.011	0.589
	Individual	1.344	-0.001	<0.001	0.981
rΔCL (mm)	Communal	<0.001	<0.001	<0.001	1.000
	Individual	0.397	-0.030	<0.001	0.943
D (day)	Communal	65.684	2.962	0.047	0.248
	Individual	-1.462	11.858	0.116	0.065

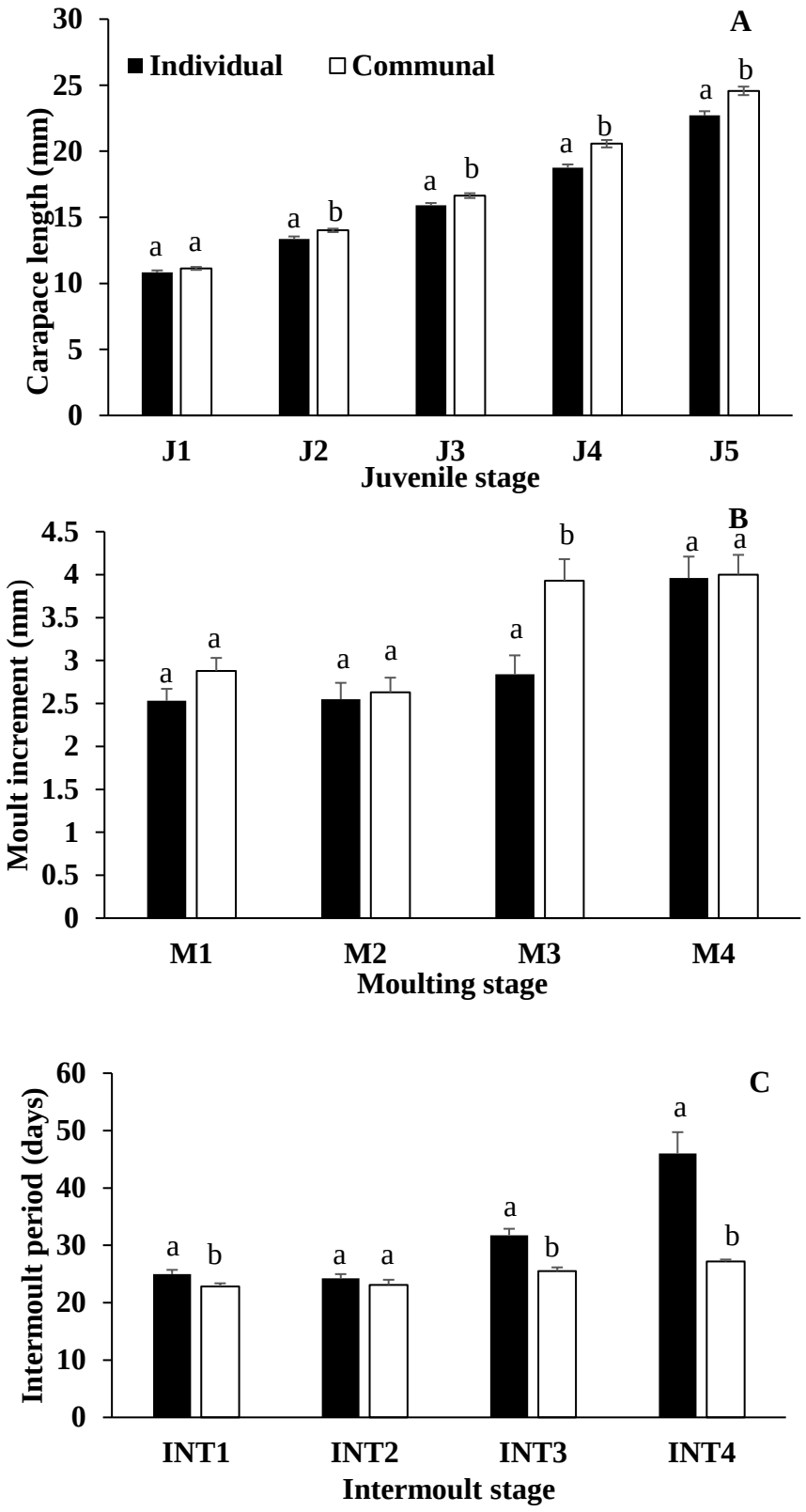

Figure 4.4 Results of the effect of *Jasus edwardsii* emergent juvenile rearing treatment experiment. (A) carapace length, (B) moulting increment, (C) intermoult period. Data are means and error bars represent standard error. Significant differences between means within the same stage of the two rearing treatments are marked by different letters. (one-way ANOVA, $P < 0.05$)

Table 4.4 Summary of three-way ANOVA analyses of intermoult period, moult increment and carapace length of *Jasus edwardsii* emergent juvenile reared individually and communally (P <0.05)

Factor	df	F	*P*
Carapace length			
Rearing treatment	1, 280	54.236	<0.001
Juvenile stage	1, 280	974.478	<0.001
Sex	1, 280	1.426	0.233
Treatment X Stage	4, 280	4.739	0.001
Treatment X Sex	1, 280	0.240	0.625
Sex X Stage	4, 280	0.633	0.640
Treatment X Stage X Sex	4, 280	0.895	0.467
Moult increment			
Rearing treatment	1, 224	6.129	0.014
Intermoult stage	3, 224	19.018	<0.001
Sex	1, 224	0.650	0.421
Treatment X Stage	3, 224	3.128	0.027
Treatment X Sex	1, 224	0.371	0.543
Sex X Stage	3, 224	2.228	0.086
Treatment X Stage X Sex	3, 224	1.364	0.255
Intermoult period			
Rearing treatment	1, 224	42.160	<0.001
Intermoult stage	3, 224	31.005	<0.001
Sex	1, 224	0.269	0.604
Treatment X Stage	3, 224	14.926	<0.001
Treatment X Sex	1, 224	0.422	0.517
Sex X Stage	3, 224	0.051	0.985
Treatment X Stage X Sex	3, 224	0.220	0.883

Table 4.5 Details of linear regression (y=a+bx) describing the relationship between moulting period (D) with residual carapace length increment (ΔCL) of *Jasus edwardsii* emergent juvenile presented in Figure. 4.5 (ANOVA, $P < 0.05$).

Treatment	A	b	r^2	*P*
Communal	-1.780	0.018	0.007	0.723
Individual	-0.470	0.004	0.005	0.658

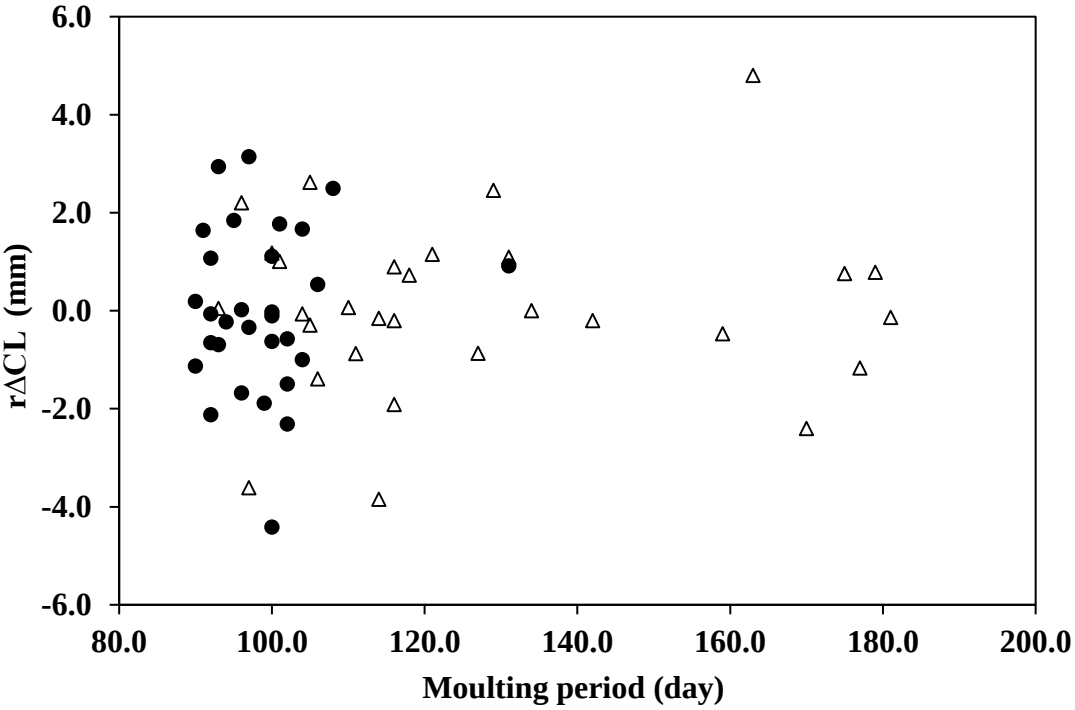

Figure 4.5 Relationship between moulting period (days) and residual carapace length increment (mm) of *Jasus edwardsii* emergent juvenile reared (●) individually or (Δ) communally. Each data point represents an individual lobster. Details of regression lines are presented in Table 4.5.

Table 4.6 Coefficient variation of intermoult period, moult increment and carapace length of *Jasus edwardsii* emergent juvenile reared individually and communally.

CV	Individual	Communal
Carapace Length		
CL_{J1}	7.41	4.97±0.91
CL_{J2}	7.18	3.98±0.64
CL_{J3}	5.80	5.93±0.60
CL_{J4}	6.99	6.94±1.58
CL_{J5}	7.78	7.04±1.30
Moult increment		
M_1	29.85	27.44±5.92
M_2	40.46	35.83±6.67
M_3	41.84	33.51±11.19
M_4	34.79	29.11±11.54
Intermoult period		
INT_1	16.45	11.15±2.64
INT_2	16.05	17.02±6.02
INT_3	19.15	13.27±2.10
INT_4	44.28	6.93±1.33

CV: coefficient variation, CL: carapace length, M: moult increment, INT: intermoult period

4.4.4 Effect of metabolic phenotype on growth

Mass-specific AMR and AS (mg O_2 h^{-1} g^{-1}) of communally reared lobsters decreased significantly ($P < 0.05$) with dry body weight (g) with larger lobsters demonstrating lower mass-specific metabolism (Table 4.7, Figure 4.6). There was no significant interaction between mass-specific SMR and RMR of both treatments with DBW.

Lobster's observed SMR, RMR, AMR and AS significantly increased linearly ($P < 0.05$) with DBW in both treatments (Table 4.8, Figure. 4.7). Residual SMR, RMR, AMR and AS (rSMR, rRMR, rAMR and rAS) for the lobsters from both rearing treatments were calculated from regression equations in Table 4.8 and distributed as in Table 4.9.

Lobster's rRMR, rAMR and rAS were positively linked ($P < 0.05$) to residual ΔCL for the individual rearing treatment whereas there was no significant ($P > 0.05$) relationship for communally reared lobsters were observed (Table 4.10, Figure 4.8). There was no significant relationship between rΔCL and rSMR in either treatments ($P > 0.05$).

Table 4.7 Details of linear regression (y=a+bx) describing the relationship between standard metabolic rate (SMR), routine metabolic rate (RMR), active metabolic rate (AMR) and aerobic scope (AS) (mg O_2 h^{-1} g^{-1}) and dry body weight (g) of *Jasus edwardsii* emergent juvenile presented in Figure 4.6 (ANOVA, P <0.05).

Treatment	Metabolic rate (mg O_2 h^{-1} g^{-1})	a	b	r^2	*P*
Individual	SMR	0.087	-0.015	0.026	0.402
	RMR	0.153	-0.025	0.049	0.251
	AMR	-0.023	0.257	0.019	0.479
	AS	0.170	-0.008	0.145	0.727
Communal	SMR	0.084	-0.003	0.008	0.638
	RMR	0.114	0.003	0.005	0.716
	AMR	0.255	-0.034	0.145	0.038*
	AS	0.171	-0.031	0.127	0.053*

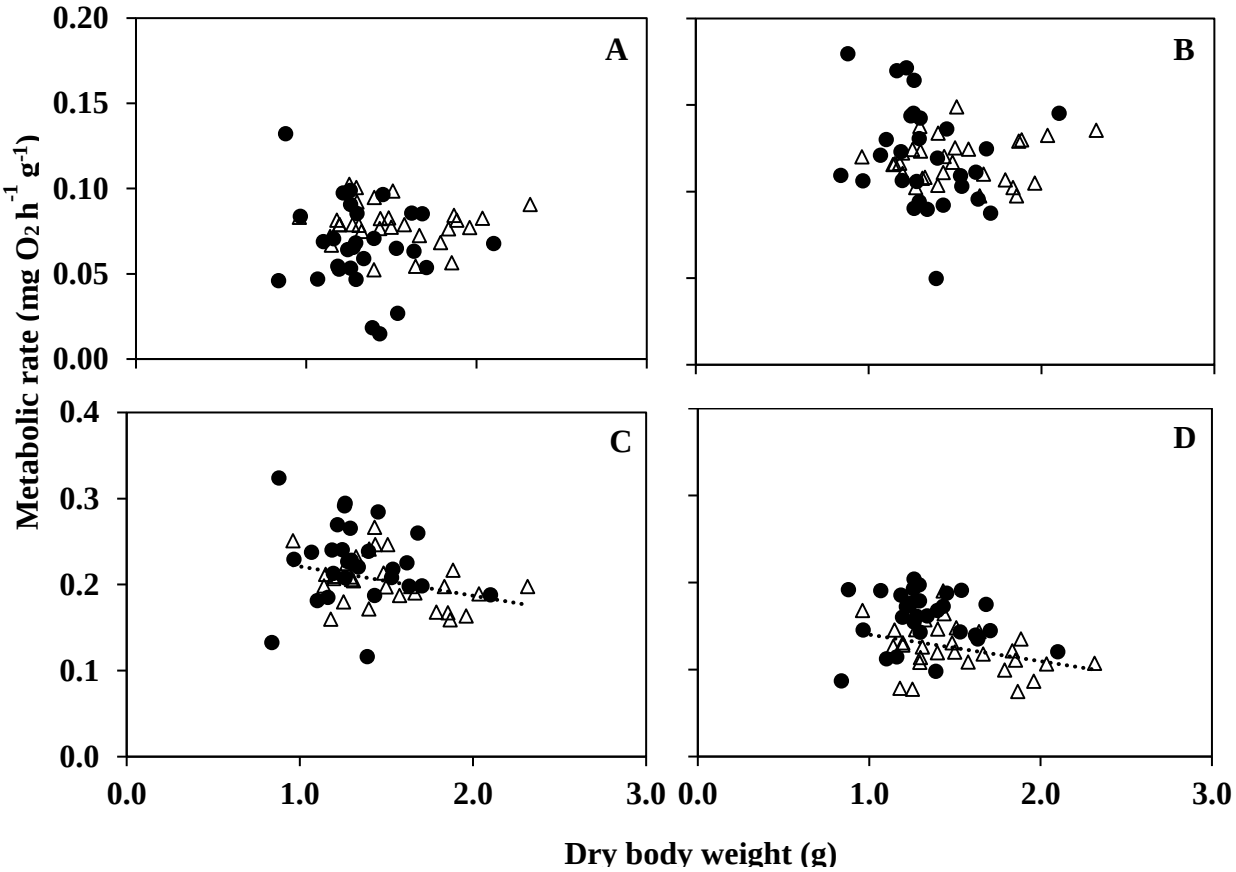

Figure 4.6 Relationship between mass-specific metabolic rate (mg O_2 h^{-1}) and dry body weight (g) of *Jasus edwardsii* emergent juvenile reared (●) individually or (Δ) communally. A; standard metabolic rate (SMR), B; routine metabolic rate (RMR), C; active metabolic rate and D; aerobic scope. Each data point represents an individual lobster. Details of regression lines (dotted lines) are presented in Table 4.7.

Table 4.8 Details of linear regression (y=a+bx) describing the relationship between observed standard metabolic rate (SMR), routine metabolic rate (RMR), active metabolic rate (AMR) and aerobic scope (AS) (mg O_2 h^{-1}) and dry body weight (g) of *Jasus edwardsii* emergent juvenile presented in Figure 4.7 (ANOVA, P <0.05).

Treatment	Metabolic rate (mg O_2 h^{-1})	a	B	r^2	*P*
Individual	SMR	0.019	0.051	0.155	0.035
	RMR	0.031	0.096	0.309	0.002
	AMR	0.059	0.180	0.431	<0.001
	AS	0.039	0.129	0.433	<0.001
Communal	SMR	0.004	0.076	0.633	<0.001
	RMR	0.126	-0.011	0.782	<0.001
	AMR	0.082	0.147	0.590	<0.001
	AS	0.078	0.071	0.272	0.003

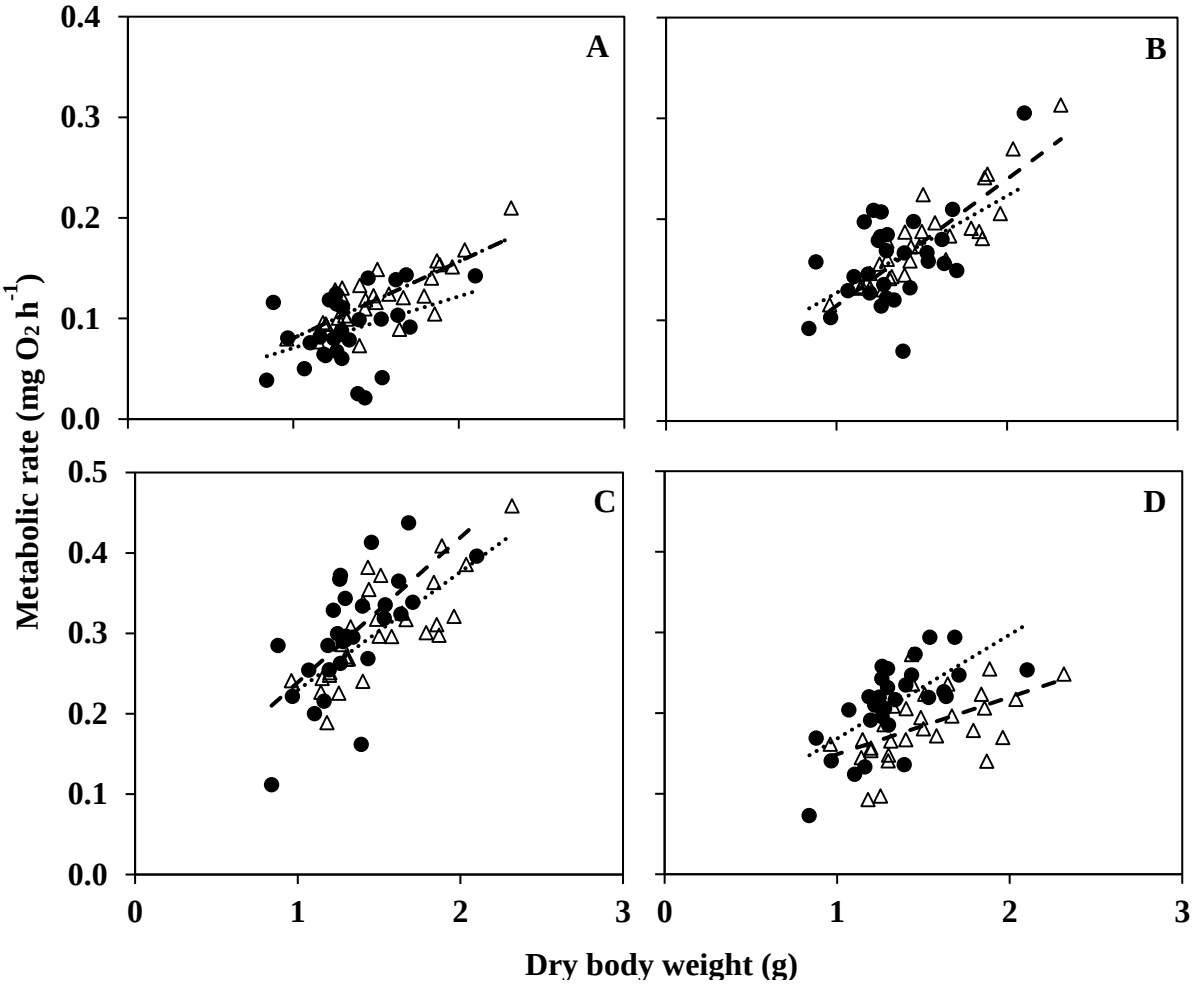

Figure 4.7 Relationship between observed metabolic rate (mg O_2 h^{-1}) and dry body weight (g) of *Jasus edwardsii* emergent juvenile reared (●) individually or (Δ) communally. A; standard metabolic rate (SMR), B; routine metabolic rate (RMR), C; active metabolic rate (AMR), D; aerobic scope (AS). Each data point represents an individual lobster. Details of regression lines are presented in Table 4.8.

Table 4.9 Distribution of residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Jasus edwardsii* emergent juvenile reared either communally or individually.

Metabolic rate (mg O_2 h^{-1})	Individual (n=29)		Communal (n=30)	
	High $\dot{M}o_2$	Low $\dot{M}o_2$	High $\dot{M}o_2$	Low $\dot{M}o_2$
rSMR	16	13	14	16
rRMR	12	17	17	13
rAMR	15	14	13	17
rAS	15	14	14	16

Table 4.10 Details of linear regression (y=a+bx) describing the between residual carapace length increment (rΔCL, mm) with residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Jasus edwardsii* emergent juvenile reared individually or communally presented in Figure 4.8 (ANOVA, P <0.05).

Treatment	Metabolic rate (mg O_2 h^{-1})	a	b	r^2	P
Individual	SMR	0.000	-0.003	0.021	0.451
	RMR	-0.001	0.012	0.192	0.018
	AMR	-0.002	0.016	0.145	0.042
	AS	-0.002	0.012	0.184	0.020
Communal	SMR	0.000	-0.002	0.022	0.478
	RMR	-0.000	-0.001	0.012	0.564
	AMR	0.000	-0.003	0.018	0.476
	AS	-0.000	-0.002	0.005	0.718

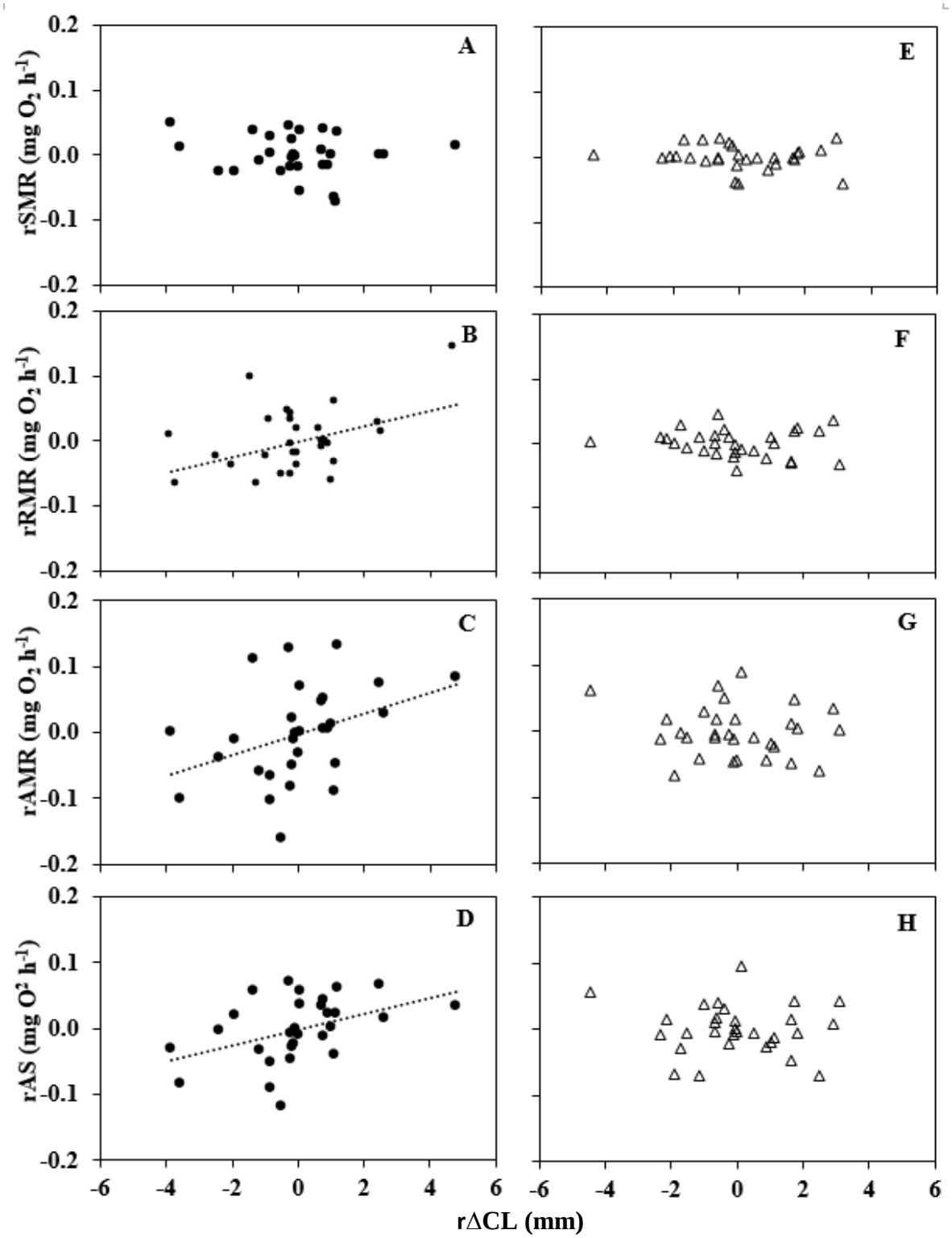

Figure 4.8 Relationship between residual carapace length increment (rΔCL, mm) with residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR,) and residual aerobic scope (rAS) (mg O_2 h^{-1}) of *Jasus edwardsii* emergent juvenile reared individually (A-D) or communally (E-H). Details of regression lines (dotted lines) are presented in Table 4.10.

4.5 DISCUSSION

Although various studies have investigated the size distribution and other factors influencing the growth of post-metamorphosis spiny lobsters, no study to my knowledge has been done to examine the influence of body size, metabolic phenotype and social behaviour on growth performance in emergent juveniles of spiny lobster. Present findings demonstrated that post-metamorphosis size had no significant influence on growth in *J. edwardsii* emergent juveniles indicating smaller size J1 can be competent and grow as fast as larger size J1. Furthermore, the study has shown a clear link between individual metabolic phenotype and growth performance of *J. edwardsii*. However, social interactions override the influence of individual metabolic phenotype on lobster growth when they are cultivated communally. Therefore, my results suggest that growth performance of emergent juvenile spiny lobsters can be directly influenced by individual metabolic phenotype but not body size at post-metamorphosis. However, social interaction plays a more important role in determining the growth of individuals.

4.5.1 Size distribution of the emergent juvenile and effect on growth

The emergent juvenile of *J. edwardsii* exhibited a great variation in size at post-metamorphosis. This finding is similar to other spiny lobster species where great variability in size has also been reported (Grobler & Ndjaula, 2001 ; Booth, 1979 ; Groeneveld et al., 2010). Intraspecific size variation at post-metamorphosis could be due to the larval experiences prior to metamorphosis (Pechenik et al., 1998; Phillips, 2002; Pechenik et al., 2002; Marshall et al., 2003; Phillips, 2004; Emlet & Sadro, 2006; Pechenik, 2006; Marshall & Morgan, 2011). In spiny lobsters, size disparity of the emergent juveniles could be influence from the variances in environmental and nutritional condition experienced by individuals during the larval development phase and might correspond to inter-individual variation in condition of emergent juveniles (Jeffs et al., 2001a ; Jeffs et al., 2001b). Previous findings showed that larger pueruli of *J. edwardsii* have larger energy reserves. This may provide a nutritional advantage for these larger larvae at inshore settlement (Jeff et al., 1999; Jeffs et al., 2001a). Whist not measured in the present study, the size variation observed among *J. edwardsii* emergent juvenile could be possibly due to differences in condition status of individual lobsters and therefore requires further investigation.

Body size of the emergent juveniles in this present study did not significantly influence growth of juvenile *J. edwardsii* in either individual or communal culture. This result

demonstrates that small sized emergent juveniles are also capable of growing as fast as large size emergent juveniles where food resources are not limited and predators excluded. This finding also refutes the hypothesis of the study that larger juveniles may display greater growth potential due to improved nutritional condition and latent effect of conditions experienced during the species long larval development. Although earlier studies on aquatic animals demonstrates that larval experiences and competency at life period transitions such as metamorphosis can have profound influences on juvenile growth performance (Phillips, 2004; De Block & Stoks, 2005; Emlet & Sadro, 2006; Giménez, 2006; Marshall & Keough, 2006; Pechenik, 2006; Giménez, 2010; Crean et al., 2011; Diederich et al., 2011), in this present study it is remains unclear why size of the emergent juvenile has little influence on *J. edwardsii* growth. This lack of result could be related to the culture condition where highly nutritious food resources are not limited and at optimum environmental conditions and thus does not reflect the emergent juvenile performance in the wild. Alternatively, the extended and complex larval life cycle of lobsters' may possibly act to exclude poorer performing individuals and only the successful are able to return to shore and settle. However, this remains an interesting possibility that needs further investigation.

4.5.2 Effect of social interaction on growth, moulting and feeding activity

Present results show that differences in growth performance between individual and communal rearing treatments as represented by difference in carapace length, moult increment and intermoult duration. This result is consistent with those demonstrated in Chapter 2 for *S. verreauxi, Panulirus ornatus* (Irvin & Williams, 2008 ; Ratunil Jr, 2017) *Panulirus cygnus* (Vijayakumaran et al., 2010) and *Panulirus homarus* (Chittleborough, 1975) where communally reared lobsters grew 11% more compared to those reared individually over just four moults.

Growth in crustaceans has been reported as a discontinuous process which is achieved by a succession of moults over time (Hartnoll, 1982). Moulting processes consist of two different components; moult increment and intermoult period (Thomas et al., 2003). In general, the intermoult period increased and moult increment decreased as the lobster's size increased, as was observed in the current study. In the present study, the differences in lobster's growth were attributed to differences in the moult increment and intermoult period. Communally reared lobsters displayed higher moult increment and shorter moulting period to moult from J1 to J5 stage than those reared individually. During the first intermoult, the moult interval was

9% shorter in communally reared lobster and it increased to 70% shorter during the fourth intermoult. Similar moulting patterns has also been displayed in *P. homarus* by Vijayakumaran et al. (2010) and *S. verreauxi* (Chapter 2). Although intermoult period was not reported, studies on *P. ornatus* by Irvin and Williams (2008) and Ratunil Jr (2017) demonstrated a similar moulting pattern since communally reared lobsters showed greater moult increment and body weight increases. Previous studies reported that lobsters are also able to recognise the odour of conspecifics such as chemical stimuli produced during moulting, agonistic and reproductive behaviours (Aiken & Waddy, 1980 ; Waddy & Aiken, 1990). The role of conspecific chemical stimuli on the moulting of lobsters remains unclear. It is possible that the longer moulting period in individually reared lobsters was influenced by a lack of chemical stimuli. Coefficient variation of moult increment, moulting duration and moulting interval of lobsters in communally rearing treatments were lower than those in individual rearing. These results indicate that with time, the moulting patterns of the lobsters in communal rearing became more uniform, which may have been influenced by conspecific chemical stimuli (Thomas et al., 2003).

Elimination of social interaction and social hierarchy provides the individual lobster unopposed access to available food and shelter which could be considered an advantage for individual reared lobster performance. However, in the present study, lobsters reared individually did not benefit from unrestricted access for food and shelter and displayed lower growth than those in communal rearing. This finding is similar to my previous observation on *S. verreauxi* where higher feeding activity and higher feed intake were observed in communal reared lobsters in comparison to individual reared lobsters (Chapter 2). Hence, the improved growth in the current study was more likely to be attributed to the resultant increased feeding activity and feed intake in communally reared lobsters. Irvin and Williams (2008) suggesting that higher growth rate observed in communal rearing lobsters may be due to the feeding stimulation through the competition for food with conspecifics. Among spiny lobsters, agonistic behaviour and the ability to form highly-structured dominance hierarchies has been observed (Fielder, 1965a ; Fielder, 1965b ; Cobb, 1981 ; McCarthy, 2000 ; Thomas et al., 2003 ; Segura-García et al., 2004; Shabani et al., 2009). With the presence of dominant individuals, it can directly and indirectly influence the growth of the subordinate individuals over food competition, appetite suppression, altered food-conversion efficiency and an increase in motor activity in the subordinate individual (Karplus, 2005). Thomas et al. (2003) reported that agonistic where the dominant individual controlled a disproportionate share of food

resources compared to subordinates which caused size disparity and growth depensation. Alternatively, Carter et al. (2014) suggested that growth depensation may reduce as the lobster grow and with adequate feeding as adult of *J. edwardsii* reared over an eight-month period did not display any growth depensation. In this present study, the CV_{DBW} and CV_{CL} were greater in communal rearing indicating that with social interaction, the growth depensation in early stages of juvenile development increases probably due to the presence of dominance hierarchies. However, the CV_{CL} of individually reared lobsters remained stable throughout the rearing experiment whereas communally reared lobsters increased over the four moults period. This indicates that growth disparity increased over time in communally rearing revealing that social interactions occur during very early juvenile stages.

In the present study, cannibalism was not observed. However, consumption of the shedded exoskeleton by conspecifics in the communal rearing treatment was observed. This finding is consistent with results demonstrated by *Homarus americanus* (Horne & Tarsitano, 2007). However, in this present study, moulted lobsters reared individually may not have consumed their own shedded exoskeletons due to recovery from demanding physiological process of moult where lobster have been shown to cease feeding and the shedded exoskeleton were removed daily in routine cleaning (Simon et al., 2015). In communal rearing, occurrence of moulting was not synchronous and non-moulting lobsters probably consumed the exoskeleton from conspecifics. To our knowledge, no studies have been conducted assessing the effects of exoskeleton consumption on the growth of lobsters. Conspecific consumption of the shed exoskeleton may provide an increased nutrient uptake and enhance lobster growth in communal rearing treatments and is an area that requires further study.

The results of the present study also show no significant interactions between growth and sex in both communal and individual rearing. This finding is in agreement with previous results on *S. verreauxi* (Chapter 2) and *Jasus lalandii* (Hazell et al., 2001). Possibly a reflection of the immature status of lobsters examined.

4.5.3 Effect of metabolic phenotype on growth

Jasus edwardsii early juveniles demonstrated a positive direct link between metabolic phenotypes (rRMR, rAMR and rAS) and growth performance in the absence of social interaction. This finding agrees with previous findings with *S. verreauxi* (Chapter 2). Hence, the results suggest that the link between aerobic metabolic phenotype and growth in the absence of social interaction is typical for spiny lobster species.

In previous research, animals with higher metabolic rate were stated to have a higher cost of maintenance, greater potential to process food which requires greater amounts of food intake to maintain their large "metabolic machinery" and resulting in more rapid growth (Hou et al., 2008 ; Van Dijk et al., 2002 ; Millidine et al., 2009 ; Biro & Stamps, 2010 ; Killen et al., 2016). Furthermore, Auer et al. (2015) also described that individuals with greater AS could consume more food and grow better compare to individuals with a lower AS when food is abundant. This is consistent with my present findings where lobsters with high AS showed a greater growth compared to lobsters with lower AS when reared individually under ad libitum conditions. In the present study, rSMR displayed no correlation with lobster's growth performance. This finding was unexpected and contradict with my previous finding on *S. verreauxi* juveniles (Chapter 2) where result suggested that there is a significant positive link between rSMR and lobsters' growth. The differences in results could be due to different species and juvenile size (~30mm CL).

Lobsters reared communally demonstrated that metabolic phenotype was not associated with growth indicating that the influence of social interaction outweighed the direct link between metabolic phenotype and growth as observed in individual rearing treatment. This result is consistent with my previous study demonstrated by *S. verreauxi* juveniles in Chapter 2. With the influence of social interaction, high SMR lobsters may not benefits from the 'increased intake' hypothesis as observed in individual rearing lobsters. There are several conditions which may influence the growth advantage of the high metabolic rate animal in communal rearing including the influence of sex, dominance and competitive ability (Brown et al., 2003 ; Álvarez & Nicieza, 2005 ; Killen et al., 2013). Previous study on *S. verreauxi* juveniles (Chapter 3) demonstrated that low metabolic rate lobsters displayed a greater ability to win over high metabolic rate lobsters in a feeding contest experiment and showed potential to become more dominant. Hence this may give an advantage to the lower metabolic rate lobster in the present study to grow as fast as high metabolic rate lobster due to their social status. Moreover, greater excess of resources can also be beneficial to the low metabolic rate lobster as it can be directed towards growth (Burton et al., 2011). Major costs in terms of either energy expended during agonistic contest or loss of feeding opportunities faced by the high metabolic rate lobsters may indirectly generate a non-significant relationship between metabolic rate and growth as observed in the present study (Turnbull et al., 1998 ; Nicieza & Metcalfe, 1999).

4.5.4 Conclusion

The results of the present study demonstrated that growth performance of *J. edwardsii* is affected by metabolic phenotypes but not body size at post-metamorphosis with social behaviour playing an important role in determining the growth of individual lobsters. The lack of relationship between emergent size and growth is contrary to the hypothesis of the study that larger juveniles may show greater growth potential due to latent effect of larval experiences which could be related to the optimal culture and nutritional conditions of the present study. Higher metabolic phenotypes can allow individual lobsters to maximize their growth under individual rearing conditions. However, correlations between energy metabolism and growth can be overridden by social interaction when lobsters are reared communally. Social interaction may also cause the individual lobsters to utilize their energy for activities such as foraging and agonistic interactions which can otherwise be used for other physiological function such as maximizing their growth. Lobsters reared communally grew significantly faster than individually rearing. This is possibly due to feeding competition with conspecific, which may cause greater growth disparity and depensation over time. Metabolic phenotype can be used as a reliable predictor for spiny lobster's growth performance in captivity. The present findings also agree with previous findings with *S. verreauxi* (Chapter 2) and suggests the link between aerobic metabolic phenotypes and growth in the absence of social interaction may be universal for spiny lobster species. Similar to the conclusion in Chapter 2 the findings of the present study demonstrate the overriding influence of social interaction on lobster growth.

CHAPTER 5

FOOD PREFERENCES AND FEED INTAKE OF THE EMERGENT JUVENILE SOUTHERN ROCK LOBSTER, *JASUS EDWARDSII* ON FRESH MUSSELS AND COMMERCIAL DIET: THE INFLUENCE OF METABOLIC PHENOTYPE ON INDIVIDUAL VARIATION IN FEEDING INTAKE

5.1 ABSTRACT

Previous studies demonstrate that differences in growth of spiny lobsters can be influenced by individual variation in metabolic physiology (metabolic phenotype) with higher metabolic phenotypes having greater increases in carapace length and moult frequency when social interaction is absent and food is abundant. However, the mechanisms linking metabolic phenotype with growth are poorly understood. Studies with teleosts suggest that the relationship between energy metabolism and growth is influenced by food availability and intake, however, the correlation between metabolic phenotype and feed intake has not been examined in spiny lobsters. The primary aim of this study was to investigate the influence of metabolic phenotype on individual variation in feed intake of emergent juvenile *Jasus edwardsii* in captivity. Moreover, the food preference amongst current best diets of the emergent juvenile was also determined through a multiple-choice experiment and correlated with individual lobster growth. The apparent feed intake (AFI) and food preference of emergent juveniles fed with three different feed; mussel gonad (MG), mussel mantle (MM) and a confidential commercial diet (MFD) were determined during first (J1) and third (J3) instar juvenile stages. The results demonstrated that metabolic phenotype was not correlated with individual feed intake and that lobster growth performance was not correlated with feed intake. Mussel gonad was the preferred food of juvenile lobsters, and food preference was not linked with individual growth performance. These results indicate that lobster feed intake and preference are not fundamental factors linking metabolic phenotype and growth performance, implying that mechanism underpinning metabolic phenotype and growth is intricate and may involve a range of intrinsic and extrinsic factors which need to be explored.

Keywords: apparent feed intake, food preference, emergent juvenile, metabolic phenotype, *Jasus edwardsii*

5.2 INTRODUCTION

Spiny lobsters are valuable and in high demand but with limited availability due to over-exploitation of wild stock (Ehrhardt & Fitchett, 2010 ; Linnane et al., 2010). To meet both the global demand and to reduce the fishing pressure on wild stocks, efforts have been made to produce spiny lobsters through aquaculture through the culture of either hatchery produced or wild caught juvenile seed stock. One of the most valuable temperate species of spiny lobster is the southern rock lobster, *Jasus edwardsii* which is also a promising candidate for aquaculture, particularly through the on-growing of wild juvenile seed (Jeffs, 2010). To date, one of the significant drawbacks to the successful production of spiny lobsters in captivity is large inter-individual variation in growth rate leading to growth disparity and ultimately reduced lobster biomass (Irvin & Williams, 2008 ; Vijayakumaran et al., 2010 ; Carter et al., 2014). *Jasus edwardsii* in particular has demonstrated large size disparity between individuals within a population due to the dissimilarity in individual growth performance (Crear et al., 2000 ; Thomas et al., 2003).

Size variation among individual spiny lobster in a population can be seen from the early life stages (Grobler & Ndjaula, 2001 ; Groeneveld et al., 2010) and, becomes more apparent as the lobster grows due to differences in individual growth rates. Early research on *J. edwardsii* suggested that the inter-individual size variation in post-puerulus could be related to differences in nutritional condition of individuals on completion of the larval stages; e.g. larger pueruli have larger energy stores (Jeffs et al., 2001a ; Jeffs et al., 2001b). Results in Chapter 4 showed that the emergent juvenile, also known as first instar juvenile (J1), of *J. edwardsii* displayed considerable differences in size ranging from 9.3 to 12.8 mm carapace length (CL) (coefficient variation, CV; 7.41%). As the emergent juveniles grew, the size variation of the individually reared fifth instar juvenile (J5) became more significant with size ranging from 18.8 to 27.4 mm CL (CV 7.78%). However, the variation in subsequent individual growth performance was not linked to the emergent juvenile size suggesting that other factors were contributing to growth variation among individuals. In Chapter 4, it was also shown that individual variation of *J. edwardsii* growth was correlated with metabolic phenotype in the absence of social interactions but the underlying mechanism linking metabolic phenotype with growth performance has yet to be defined.

Previous studies have shown that the relationship between energy metabolism and fitness components such as growth, behaviour, reproduction and survival in aquatic ectotherms could be highly rely on the food availability (Burton et al., 2011 ; Auer et al., 2015c). Having

a high standard metabolic rate (SMR) or high aerobic scope (AS) phenotype is often beneficial when food is abundant or easily accessible (Millidine et al., 2009 ; Auer et al., 2015a ; Auer et al., 2015b) but not when food availability is low (Killen et al., 2016a). Moreover, individuals with higher SMR or AS may be able to take advantage of high food abundance due to their ability to digest a meal more quickly, which implies they may be able to consume more food per day (Millidine et al., 2009). For example, *Salmo trutta* with a higher AS were able to ingest more food per day relative to individuals with a lower AS (Auer et al., 2015a). Additionally, a study on rainbow trout, also demonstrated that fish with high growth rates also had higher SMR, larger gastrointestinal tracts, maximum food consumption and better growth efficiency (Allen et al., 2016). Collectively, findings from previous studies show that the relationship between energy metabolism and growth in fish is influenced by food availability and intake.

Spiny lobsters are known as "picky eaters" as they are selective towards their preferred food (Eurich et al., 2014 ; Williams, 2009 ; Williams et al., 2005). Previous research has demonstrated that emergent juvenile spiny lobsters improved their food consumption and growth performance when fed mussels compared to artificial diets such as dry pellet (Tsvetnenko et al., 1999 ; Williams et al., 2005 ; Dubber et al., 2004 ; Simon & James, 2007). This is likely due to greater attractiveness and stability of mussel compared to artificial diets which quickly lose their attractiveness after immersion in the water because of high nutrient leaching which may cause the loss of chemoattractants (Glencross et al., 2001 ; Tolomei et al., 2003 ; Williams et al., 2005). Understanding lobster feeding preference is important in order to improve their feeding performance (e.g. feeding intake, feeding capacity) which indirectly can improve their growth performance.

The first aim of the present study was to examine the effect of metabolic phenotype on individual feed intake and growth performance of the emergent juvenile of *J. edwardsii*. The second aim was to identify the food preference amongst current best diets of the emergent juveniles using multiple-choice feeding experiment and linked with individual growth performance. This experiment was conducted by determining the apparent feed intake (AFI) and carapace length increment of the first instar (J1) and third instar (J3) juvenile. Food preferences were evaluated by feeding the emergent juveniles with three different feeds; fresh blue mussel, *Mytilus galloprovincialis* gonad (MG), mussel mantle (MM) and a confidential commercial diet (identified as moist formulated diet, MFD). This experimental design provides an opportunity to assess the hypothesis whether lobster feed intake can be predicted from metabolic phenotype where social interaction is absent and food availability is unrestricted,

and to assess if lobster food preference affects their growth performance. Results achieved from this study will provide useful insight into the mechanisms influencing the relationship between lobster growth and physiological traits and, suitable diet to promote optimal growth of the emergent juvenile based on their food preference.

5.3 MATERIALS AND METHODS

5.3.1 Experimental animals

Wild pueruli (at the transparent and pigmented stages) of *J. edwardsii* were caught from October to December 2016 from three coastal locations; Bicheno (41.8713° S, 148.3024° E), Recherche Bay (43.5938° S, 146.9187° E) and South Arm (43.0530°S, 147.4169°E) Tasmania, Australia using crevice collectors similar in design to that described by Booth and Tarring (1986). Live pueruli were transported to the Institute of Marine and Antarctic Studies, Taroona on the same day of collection. Collected pueruli were then communally reared in a plastic vessel (55 cm length X 21 cm width X 12.5 cm height) which received flow-through filtered sea water at three exchanges per hour. Pueruli were maintained at 18^{o}C with a light regime of 16 h light and 8 h dark until they moulted to post-pueruli (first instar juvenile (J1). Food was not provided to the lecithotrophic puerulus stage.

5.3.2 Feeding experiment

The apparent food intake (AFI) and feeding preferences of *J. edwardsii* emergent juveniles were examined through a multiple-choice experiment, in which three different diets were simultaneously offered to the lobsters. Each lobster's feeding preference was examined by calculating their AFI at two juvenile stages; the first (J1) and third (J3) juvenile instar. The correlation between AFI and growth was calculated. Growth was measured by quantifying carapace length increment (ΔCL).

A total of 29 emergent juveniles (10.83±0.80 mm carapace length (CL) were selected from the holding vessels and stocked individually into the experimental system once the lobsters moulted to J1 stage. The experimental system consisted of four rectangular plastic vessels (55 cm length X 21 cm width X 12.5 cm height) which were equally divided into eight compartments (6.8 cm length X 21 cm width X 12.5 cm height). Each of the compartments was isolated and individually supplied with filtered seawater using a flow-through system at three exchanges per hour under constant temperature (18^{o}C), salinity 33-35, pH 8.1, 80-100%

oxygen saturation and 12:12 photoperiod. A single 6 cm length cylindrical shelter made from 25 mm diameter polyvinyl chloride (PVC) pipe was placed in each compartment.

Each lobster was fed in excess of their daily feed uptake once per day with three different feeds; MFD, MG and MF. MG and MM were prepared individually by removing the organ from the mussel, rinsed with fresh water, and cut into approximately 2 cm^2 particles for MG and 2 cm length for MM. Before feeding, all the diets were weighed to 0.001 g and recorded before being placed together into the rearing vessels. One control vessel without lobsters, and under the same experimental conditions, was maintained to allow measurement of the feed lost into the water through leaching.

Remaining uneaten food was removed 24 h after feeding, pooled in weekly separate samples according to the type of diets for each replicate and frozen at -20^oC (Week 1: day 1 to 7, Week 2: day 8-14, Week 3: day 15-21). Frozen uneaten food samples were thawed and filtered using filter paper (pore size 20-25 μm). The filter paper and filtrate were then oven dried at 105^oC for 24 hour and dry matter was measured (dry weight of the food calculated by subtracting the weight of the filter paper). The difference between the dry matter weight (DW) of the food in control and replicates were used to calculate the lobster AFI (gDW individual $lobster^{-1}$ d^{-1}) as described by Fitzgibbon et al. (2017).

To assess if the individual growth performance of *J. edwardsii* emergent juveniles related to their AFI and food preference, the AFI of J1 and J3 lobsters were calculated during their instar. Lobster growth was examined by recording their CL (to determine the carapace length increment, ΔCL). Carapace length was measured using digital images taken from individual lobsters using a camera (Canon G12). Digital images were taken once the moulted lobsters had hardened sufficiently (approximately 72 hours post moult). ImageJ (Image Processing and Analysis in Java, https://imagej.nih.gov/ij/) software was used to measure the CL from the captured image. Carapace length was measured along the dorsal line from the rostrum to the dorsal margin of the carapace. Images were calibrated against images with known values (scales) and then applied to measure images at the same magnification. The experiment was terminated after all the lobsters moulted to J4 stage.

5.3.3 Oxygen consumption rate ($\dot{M}o2$)

The lobster's oxygen consumption rate ($\dot{M}o_2$) were measured using automated intermittent flow-through respirometry similar to that described by Fitzgibbon et al. (2014) after the completion of the growth experiment (Chapter 4). The respiratory system consisting of four 50 ml polypropylene respiratory chambers (50 ml conical bottom tubes) with an internal diameter of 30 mm and a length of 115 mm submerged in a water bath (24cm height x 24 cm length and 25 cm width). The respiratory system received a continuous 18^{o}C seawater supply at a rate of 17 l h^{-1}. Two twin channel mini peristaltic pumps (Harvard MP II mini peristaltic pump) were used to continuously circulate water at a rate of 10 ml min^{-1} through the chambers and past an oxygen sensor. Dissolved oxygen was recorded and logged every 20 s by a fibre optic oxygen microsensor meter (OXY-4 mini, www.preSense.de) connected to a computer. To introduce new water from the external water bath into the chamber, another two twin channel peristaltic pumps were used, and water was circulated at a rate of 14 ml min^{-1}. The pump was connected to a digital recycler timer (Sentinel DRT-1) and was programmed to turn on in 10 min and 5 min off cycles which allowed a $\dot{M}o_2$ measured every 15 min. To maintain the dissolved oxygen concentration in the external water bath at 100% saturation, an aquarium air stone was used to supply air. To exclude light and external stimuli, a black corflute screen was used to enclose the water bath housing. Throughout the $\dot{M}o_2$ measurements, dissolved oxygen within the respiratory chambers never fell below 85%. Following each experiment, the respiratory system was sterilized with a 1 mg l^{-1} solution of sodium hypochlorite, rinsed with fresh water and air dried.

The $\dot{M}o_2$ measurements were taken during the intermoult phase which was defined from day 7 to 15 post-moult. Before $\dot{M}o_2$ measurement, the lobsters were starved for 24 hours to clear the digestive tract of food and faeces and eliminate the variability of measurements associated with thermic effect of food (total energy expenditure above the SMR due to the cost of processing food for use and storage) or also known as specific dynamic action (SDA). In the late evening after 24 h of starvation, individual lobsters were placed into the respirometer chambers and $\dot{M}o_2$ logged overnight for 16 h (approximately 64 $\dot{M}o_2$ measurements). Standard metabolic rate (SMR) was defined from the mean of the lowest five recording of the $\dot{M}o_2$. The average of all the 64 recordings was defined as routine metabolic rate (RMR). To stimulate active metabolic rate (AMR), lobsters were removed from the respirometer chamber and made to swim by encouraging the lobster by hand to swim inside a small tub (40cm height X 60 cm length and 30xm width) until the lobster became exhausted and non- responsive to stimuli

(approximately 10 min) as described by (Fitzgibbon et al., 2014). Lobsters were then transferred back into the chamber, and $\dot{M}O_2$ were recorded for 2 h. Exhaustion protocol was maintained to keep in time with the open cycle of the respirometer system to allow immediate $\dot{M}O_2$ measurements. AMR was defined based on the highest 10% recordings of the $\dot{M}O_2$ measured after the exhaustion exercise. Aerobic scope (AS) was determined by subtracting the SMR from the AMR. As a measurement of background respiration, the oxygen demand of the respirometer system was then recorded for another 2 h. The lobster $\dot{M}O_2$ were determined using linear regression on the rate of decline of dissolved oxygen concentration over the final 4 min of each 5 min respirometer closed cycle period. When the R^2 of the linear regression was below 0.95, data for the period were excluded from analysis. Lobsters' mass-specific $\dot{M}O_2$ were expressed in mg O_2 h^{-1} g WW^{-1} after the subtraction of mean background respiration. Following measurements of $\dot{M}O_2$, all lobsters were removed from the chambers, and the whole-body wet weight (WW, g) was measured after removing excess moisture with paper towel.

5.3.4 Data analysis

To explore homogeneity of variance and normality of the collected data, all data were tested using Levene's test and the Shapiro-Wilk test, respectively. All lobsters were treated as individual replicates (n=29). The individual AFI of J1 and J3 lobsters were compared using Student's *t*-test to investigate the relationship between individual AFI and lobster growth (ΔCL).

Mass-specific $\dot{M}O_2$ data were expressed as residual metabolic rates (rSMR, rRMR, rAMR and rAS) calculated from linear regression of observed $\dot{M}O_2$ on body weight as described by Metcalfe et al. (1995). For this experiment data, unlogged plot was used as it was a better fit and appropriate when using a small mass range of individuals (Metcalfe et al., 1995). Residual (body weight corrected) $\dot{M}O_2$ ($r\dot{M}O2$) were determined from the regression line (the difference between observed $\dot{M}O_2$ and expected $\dot{M}O_2$). The lobsters with higher rates of oxygen consumption than that expected for their size had positive values of $r\dot{M}O_2$ while those with lower respiration rates than expected had a negative. The relationship was compared between individual AFI with rSMR, rRMR, rAMR and rAS using linear regression analysis. Due to one mortality during the $\dot{M}O_2$ measurement all statistical analyses for $r\dot{M}O_2$ were performed based on data collected from 28 lobsters.

To investigate the relationship between AFI and growth, residual carapace length increment (rΔCL) was used to estimate individual lobster ΔCL by controlling for body size due

to the range of size and CV among J1 and J3 juveniles (Secor & Dean, 1989; Hare & Cowen, 1995). Residual carapace length increment was determined from the difference between observed and expected ΔCL. Expected ΔCL was achieved by calculating the linear regression of observed ΔCL on initial CL (CL J3 stage). Lobsters with a higher value of observed ΔCL than the expected for their size had positive values of rΔCL while those with a lower value of observed ΔCL than expected had a negative rΔCL. Linear regression was performed to examine whether AFI described individual variation in growth (rΔCL).

To test the lobster food preference, two-way ANOVA and Tukey's HSD post hoc test were performed with AFI as the dependent variable and, type of diet and, time (week) as the independent variables (fixed factors). Linear regression was performed to examine whether AFI on three types of diet correlated with individual variation in growth (rΔCL). All analyses were performed using the IBM SPSS Statistics version 22.0. The level of significance for all analyses was determined at $P<0.05$. Values were presented as mean ± standard error (S.E) unless stated otherwise.

5.4 RESULTS

5.4.1 Apparent feed intake

a) Apparent feed intake by juvenile stage

Apparent feed intake varied significantly between juvenile stages: J1 lobsters consumed an average of 0.019 ± 0.002 g DW per day (*t*-test, T = 12.930, df = 28, $P < 0.001$) with J3 lobsters consuming significantly more at an average of 0.042 ± 0.002 g DW per day (*t*-test, T = 20.786, df = 28, $P < 0.001$).

b) Apparent feed intake by metabolic phenotype and growth

There was no significant relationship between rSMR, rRMR, rAMR and rAS on AFI at the J1 and J3 instar juvenile stage (Figure 5.1 and 5.2, Table 5.1). Residual carapace length increment (rΔCL) was not significantly linked with AFI for either J1 (ANOVA; F= 0.416, df = 1, 27, $P = 0.524$) or J3 (ANOVA; F= 0.350, df = 1, 27, $P = 0.559$) juvenile stages (Figure 5.3).

5.4.2 Food preference by type of feed and experimental period

The calculation for leaching and moisture content were measured from the control vessels, and MG (85%) demonstrated the greater percentage of moisture and leaching followed by MM (82%) and MFD (79%). There was no significant interaction between different type of feed and experimental period (week) for the food preference of J1 (two-way ANOVA; F= 1.694, df = 4, 252, P = 0.152) and J3 lobsters (two-way ANOVA; F= 1.255, df = 4, 252, P = 0.288) (Table 5.2).

The food preference of J1 (two-way ANOVA; F= 12.154, df = 2, 252, $P < 0.001$) and J3 stage lobsters (two-way ANOVA; F= 12.109, df = 2, 252, $P < 0.001$) were significantly influenced by type of feed. Tukey's HSD post hoc test showed that J1 lobster ingested significantly more MG than MFD in week 1 and more than both MG and MM in week 2 (Figure 5.4 A). Third instar juvenile lobsters ingested significantly more MG than MM and MFD in week 1 and more MG and MM than MFD in week 2 (Figure 5.4 B). No significant different in feed preference was observed in Week 3 for both juvenile stages.

The total feed intake of J1 stage lobsters was significantly influenced by experimental period (week) (two-way ANOVA; F= 8.688, df = 2, 252, $P < 0.001$). Tukey's HSD post hoc test showed the total feed intake of J1 juvenile stage at Week 3 was significantly higher than Week 1 but not significantly different from Week 2 (Figure 5.5). Third instar juvenile lobsters total feed intake were not significantly influenced by the experimental period (week) (two-way ANOVA; F= 1.466, df = 2, 252, P = 0.233) where results showed that the total feed intake between each week was not significantly different.

5.4.3 Effect of food preference on growth

The lobster food preference for three different feed types showed no relationship with the individual growth performance (Figure 5.6, Table 5.3).

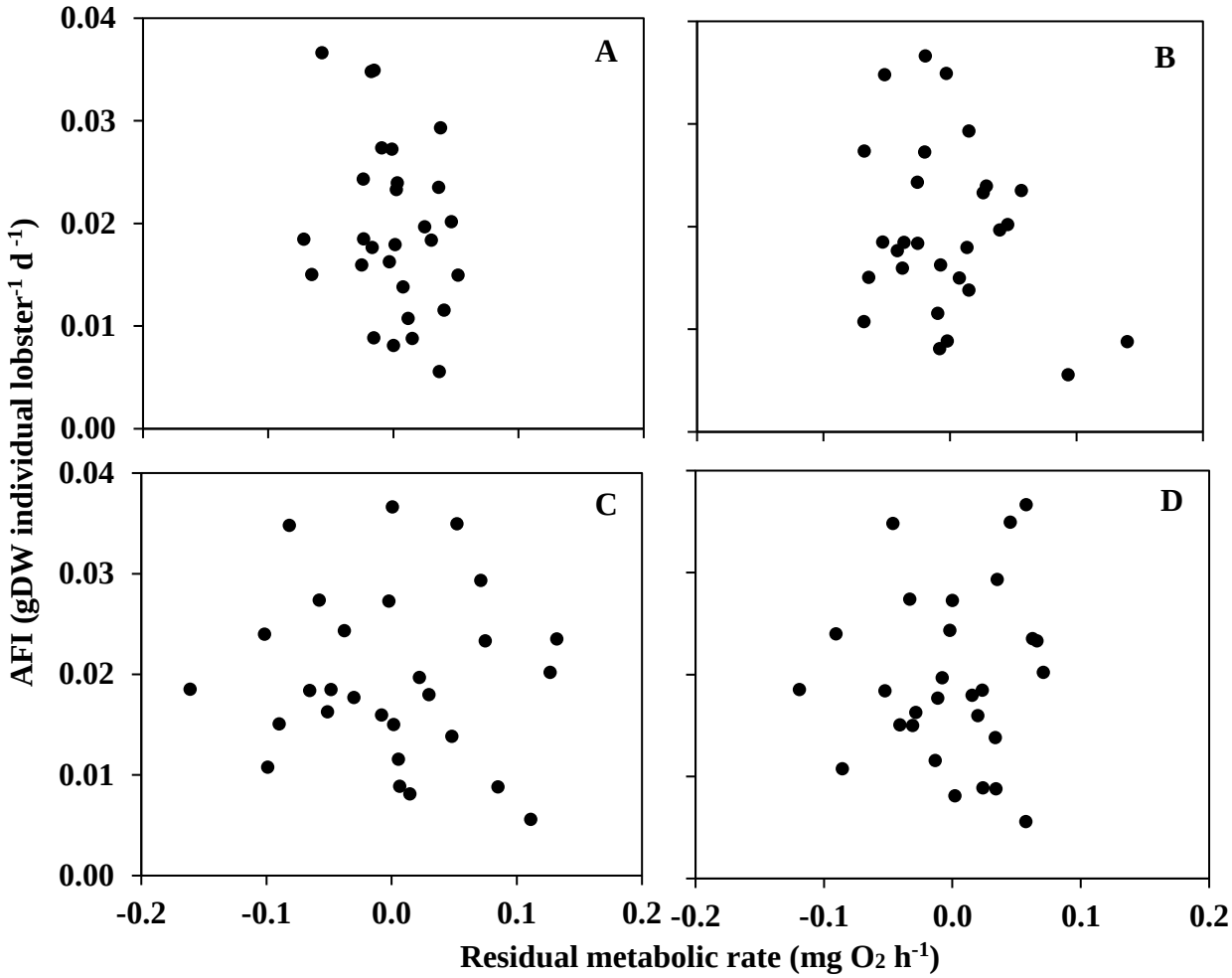

Figure 5.1 Relationship between apparent feed intake (AFI) and residual metabolic rate; (A) residual standard metabolic rate (rSMR); (B) residual routine metabolic rate (rRMR); (C) residual active metabolic rate (rAMR) and; residual aerobic scope (rAS) of *Jasus edwardsii* first instar juveniles (J1). Each data point represent an individual lobster. Details of linear regression are presented in Table 5.1

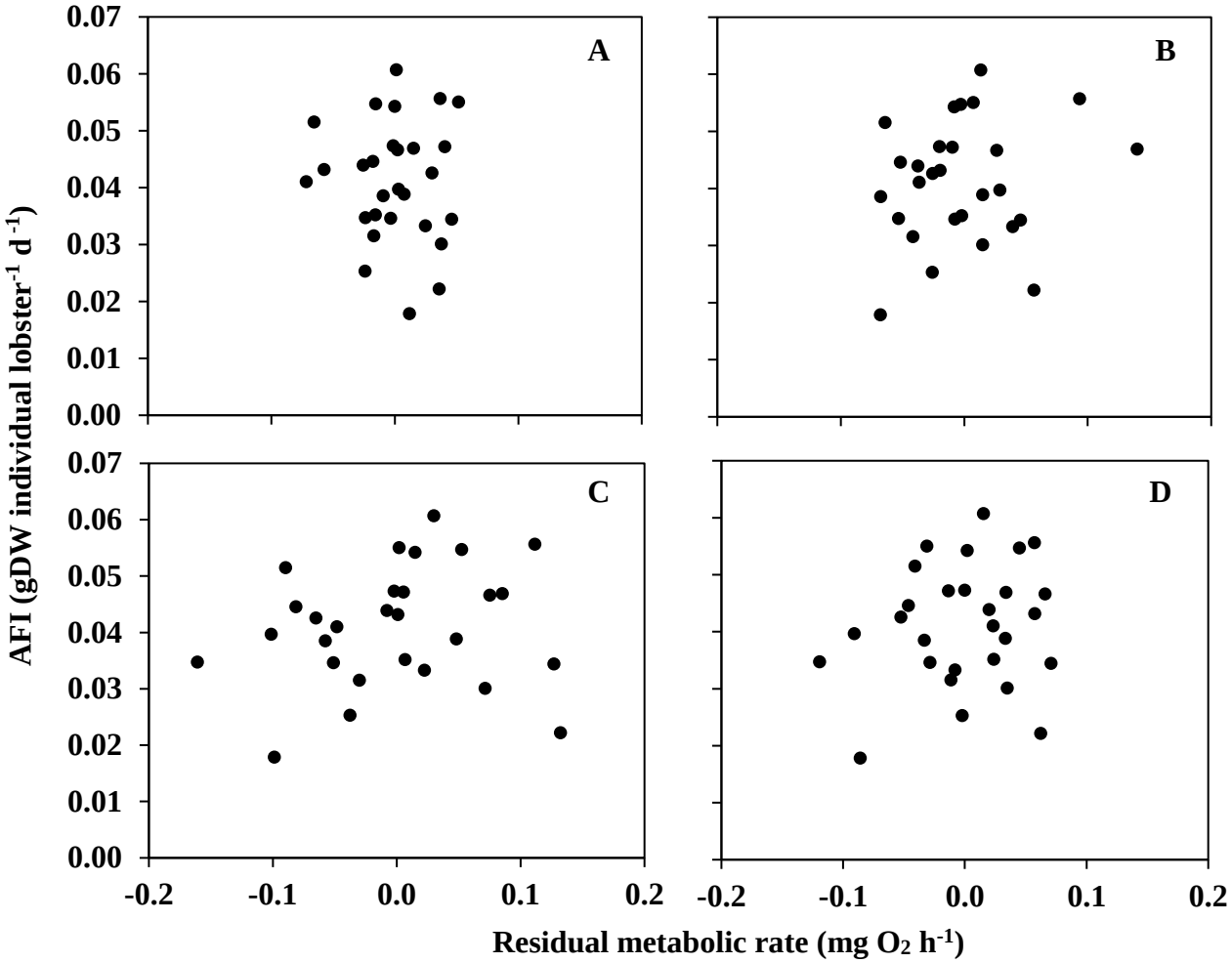

Figure 5.2 Relationship between apparent feed intake (AFI) and residual metabolic rate; (A) residual standard metabolic rate (rSMR); (B) residual routine metabolic rate (rRMR); (C) residual active metabolic rate (rAMR) and; residual aerobic scope (rAS) of *Jasus edwardsii* third instar juveniles (J3). Each data point represent an individual lobster. Details of linear regression are presented in Table 5.1

Table 5.1 Details of linear regression (y=a+bx) describing the relationship between apparent feed intake (AFI) and residual standard metabolic rate (rSMR), residual routine metabolic rate (rRMR), residual active metabolic rate (rAMR) and residual aerobic scope (rAS) of *Jasus edwardsii* first instar juveniles (J1) third (J3) instar juveniles presented in Figure 5.1 and 5.2.

Juvenile stage	Metabolic phenotype	a	b	r^2	*P*
J1	rSMR	0.020	-0.065	0.065	0.191
	rRMR	0.020	-0.045	0.070	0.174
	rAMR	0.020	-0.008	0.005	0.712
	rAS	0.020	0.014	0.007	0.680
J3	rSMR	0.036	0.006	0.000	0.943
	rRMR	0.036	0.059	0.079	0.147
	rAMR	0.036	0.033	0.058	0.223
	rAS	0.036	0.062	0.095	0.116

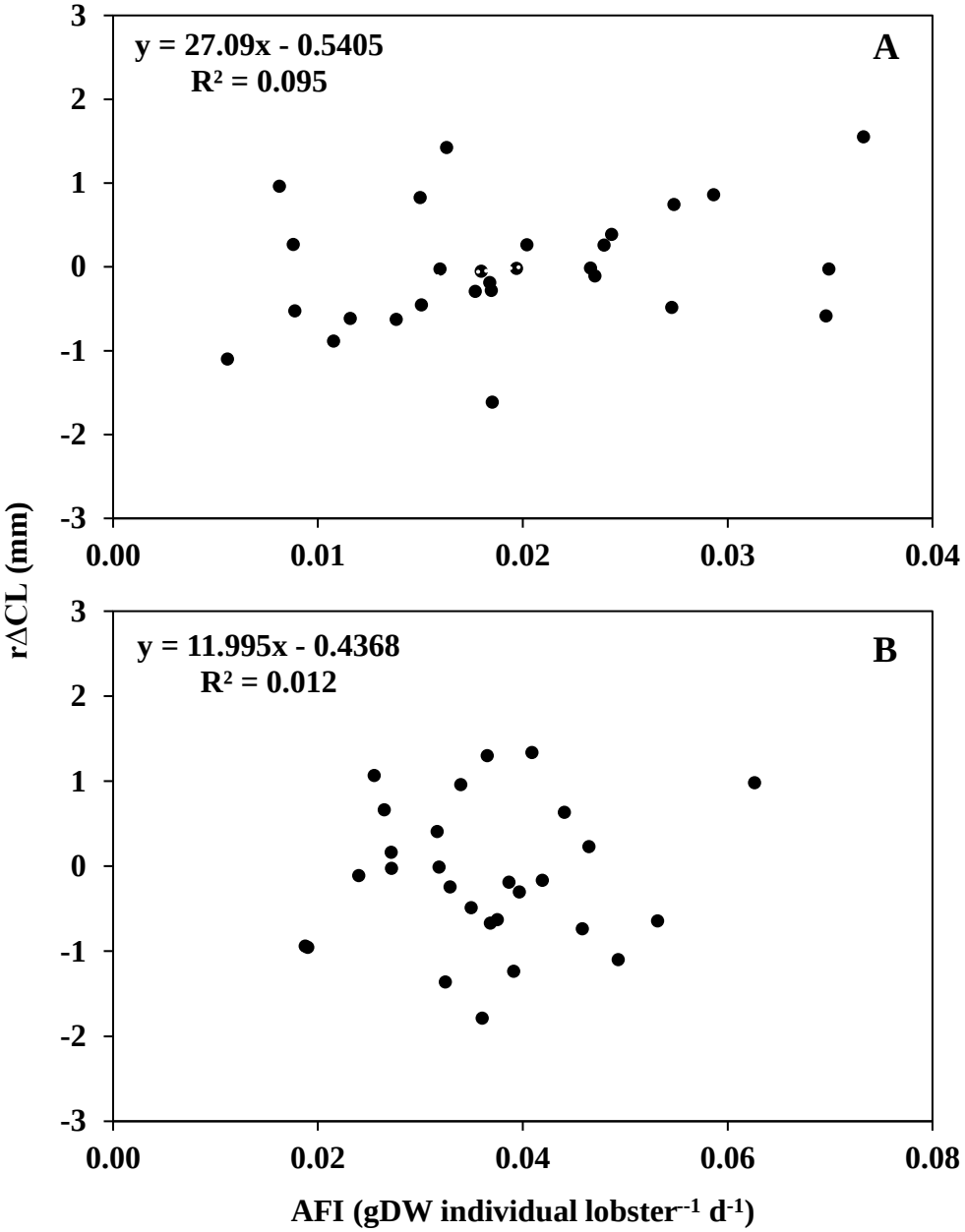

Figure 5.3 Relationship between apparent feed intake (AFI) and residual carapace length increment (rΔCL) of *Jasus edwardsii* (A) first (J1) and (B) third (J3) instar juveniles. Each data point represent an individual lobster.

Table 5.2 Two-way ANOVA results of total feed intake by *Jasus edwardsii* first instar juvenile (J1), and third instar juvenile (J3) fed with three types of diet; moist formulate diet (MFD), mussel gonad (MG) and mussel mantle (MM) (significance level P <0.05).

Juvenile stage	df	F	P
J1			
Type of diet	2, 252	12.154	<0.001*
Week	2, 252	8.688	<0.001*
Week X Diet	4, 252	1.694	0.152
J3			
Type of diet	2, 252	12.109	<0.001*
Week	2, 252	1.466	0.233
Week X Diet	4, 252	1.255	0.288

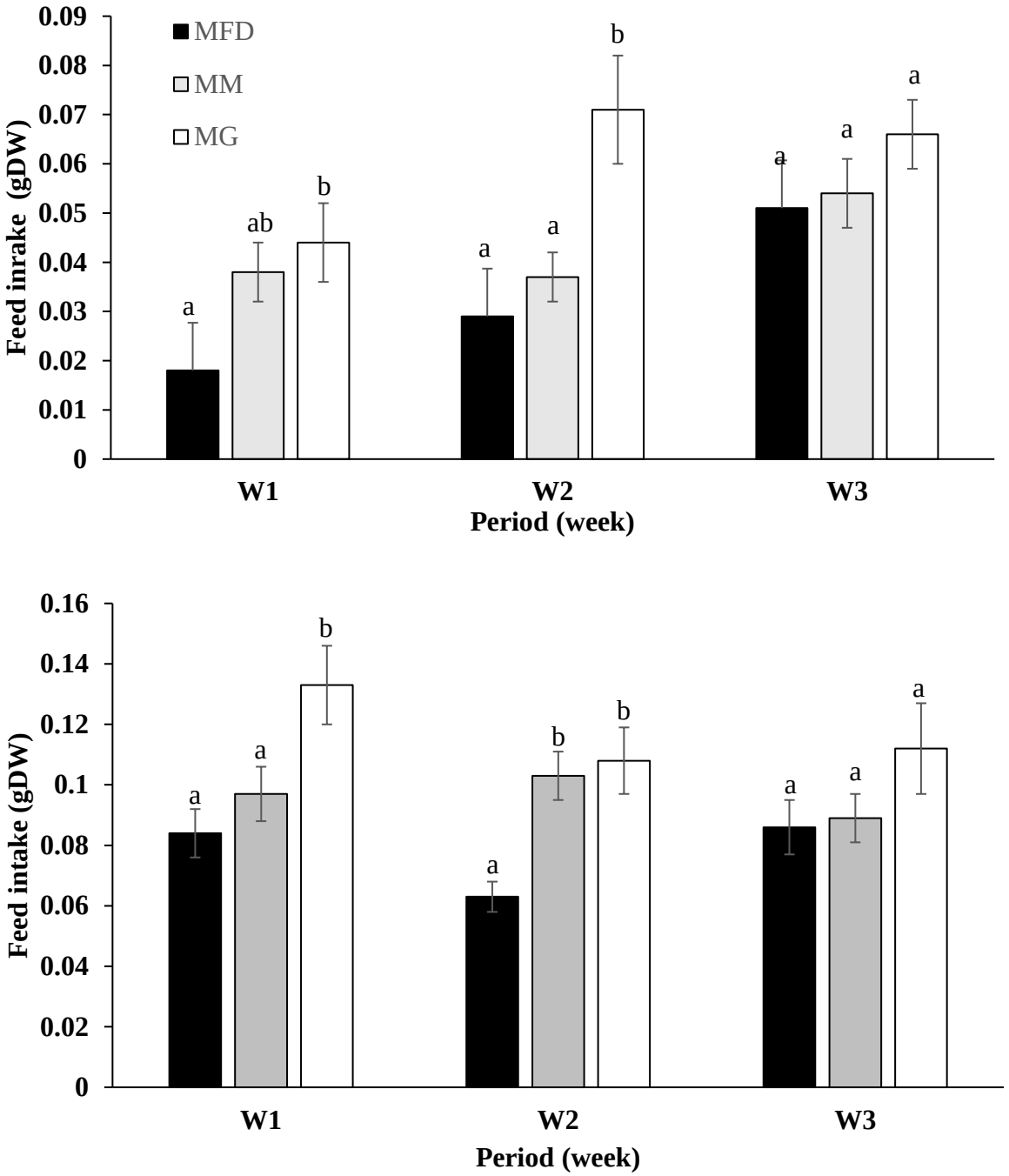

Figure 5.4 Total feed intake of *Jasus edwardsii* juveniles at (A) first instar juvenile (J1) and; (B) third instar juvenile (J3) fed with three types of diet; moist formulate diet (MFD), fresh blue mussels (*Mytilus galloprovincialis*) gonad (MG) and mantle (MM) for the period of 21 days (3 weeks). Bar bearing superscripts are significantly different between types of diet at the week indicated. Values are mean (± S.E.).

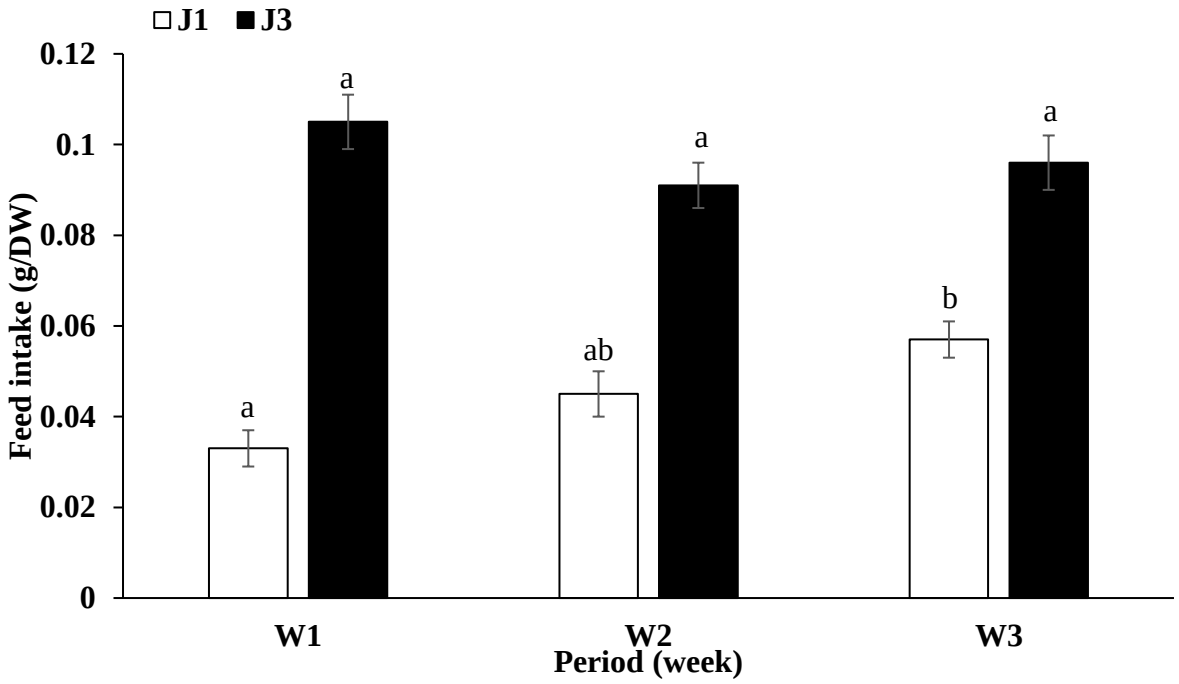

Figure 5.5 Total feed intake of *Jasus edwardsii juveniles at first* instar juvenile (J1) and third instar juvenile (J3) for the period of 21 days (3 weeks). Bar bearing superscripts are significantly different between types of diet at the week indicated. Values are mean (± S.E.).

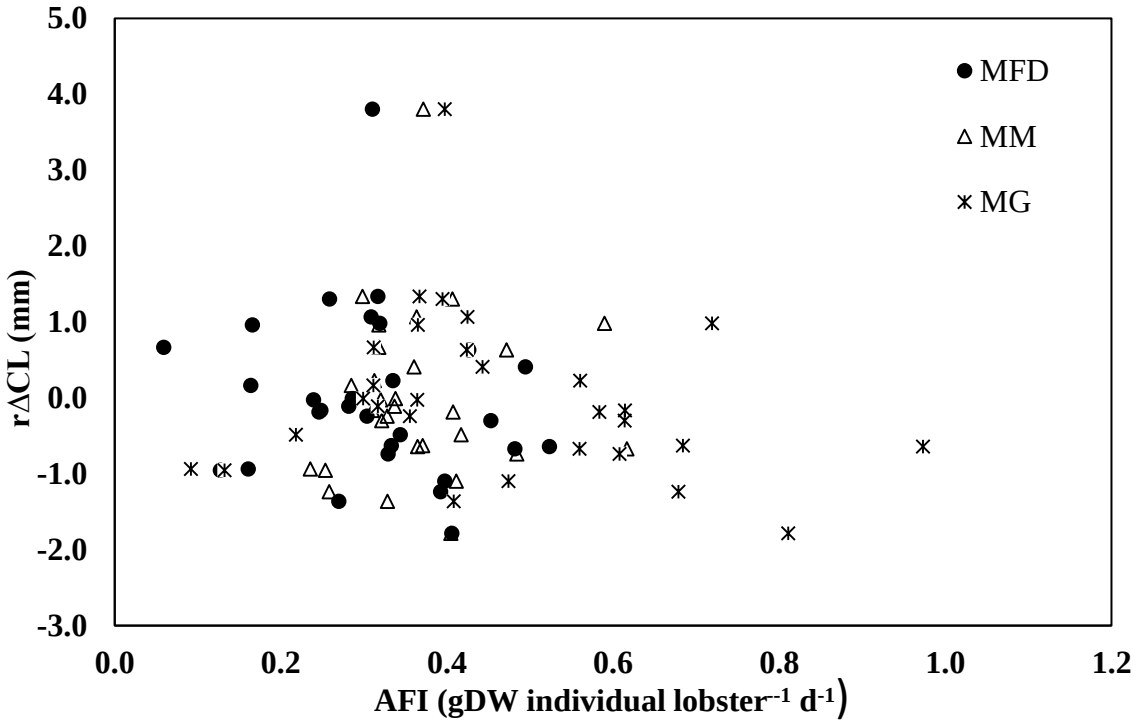

Figure 5.6 Relationship between apparent feed intake (AFI) of three types of diet; mussel gonad (MG), mussel mantle (MM) and moist formulated diet (MFD) and residual carapace length increment (rΔCL) of *Jasus edwardsii* third instar juveniles (J3). Each data point represent an individual lobster. Details of linear regression are presented in Table 5.3

Table 5.3 Details of linear regression (y=a+bx) describing the relationship between apparent feed intake (AFI) of three types of diet; mussel gonad (MG), mussel mantle (MM) and moist formulated diet (MFD) and residual carapace length increment (rΔCL) of *Jasus edwardsii* third instar juveniles (J3) presented in Figure 5.6.

Type of diet	a	b	r^2	*P*
MM	-0.339	0.926	0.006	0.701
MG	0.513	-1.103	0.040	0.301
MFD	0.474	-1.533	0.024	0.420

5.5 DISCUSSION

5.5.1 Apparent feed intake

This study has shown that individual variation in AFI of the emergent juveniles was not associated with their metabolic phenotype. Additionally, no relationship between lobster rΔCL and AFI was demonstrated indicating feed intake may not be the main influence in determining the growth of emergent spiny lobster in the absence of social interaction and when food availability was unrestricted. The current findings are consistent with previous results shown by *S. verreauxi* juveniles in Chapter 2 where AFI was not significantly linked with metabolic phenotype. It is presently unclear why AFI was not correlated to metabolic phenotype since it might be expected that animals with higher metabolic rate would need to consume more food to compensate for the increased cost of maintenance metabolism as described in previous studies (Millidine et al., 2009 ; Biro & Stamps, 2010 ; Metcalfe et al., 2016).

Findings in Chapter 4 demonstrate that the differences in growth of emergent juvenile *J. edwardsii* were influenced by individual variation in metabolic phenotype with higher metabolic phenotypes individuals having greater increase in carapace length and moult frequency when social interaction was absent and food was in abundance. This finding was similar to previous research on fish where results demonstrated that animals with higher metabolic rate tend to grow faster in high food environments (Metcalfe et al., 2016 ; Rosenfeld et al., 2015). Animals with higher metabolic rate have a higher cost of maintenance and thus require greater amounts of food consumption to uphold their large "metabolic machinery" (Van Dijk et al., 2002 ; Hou et al., 2008 ; Millidine et al., 2009 ; Biro & Stamps, 2010 ; McKenzie et al., 2015 ; Auer et al., 2015c ; Killen et al., 2016b ; Allen et al., 2016). Furthermore, studies on juvenile brown trout, *S. trutta*, show that individuals with higher AS could consume more food per day compared to fish with a lower AS (Auer et al., 2015a). However, in my study, individual AFI was not correlated to metabolic phenotype. This finding indicates that higher metabolic phenotype lobster may not require greater amounts of food to uphold their larger maintenance costs but may alternatively have greater assimilation efficiency compared to lower metabolic phenotype lobster. Previous research has shown that the variation in metabolic phenotype among *Salmo salar* is linked to their food assimilation strategy where individuals with a high SMR can process and assimilate food rapidly (Millidine et al., 2009). Furthermore, study on rainbow trout also demonstrated that fish growth rates are not only linked with their metabolic phenotype but also generally linked with the size of gastrointestinal tracts, maximum feeding capacity and growth efficiency indicating that individual growth can be influenced by

a range of integrated physiological and anatomical traits (Allen et al., 2016). Further research is needed to determine the mechanism involved in the relationship between individual metabolic phenotype and feeding efficiency and growth of lobsters in captivity.

The apparent lack of relationship between AFI and metabolic phenotype and rΔCL in the present study is possibly attributed to the probability of inaccuracies in AFI measurement in relation to spiny lobster's feeding behaviour. Spiny lobsters are considered as "messy feeders" where food is handled externally prior to ingestion. Lobsters will tear, pull, and grind apart the food particles from the food mass using their mouth part which can result in significant loss of small particles of feed (Barker & Gibson, 1977 ; Zoutendyk, 1988 ; Guillaume & Ceccaldi, 2001). Feeding losses may be influenced differently according to both ration and type of food (Thomas et al., 2002). In the present study, small particles of food were observed when the lobsters ingested the food which may have been lost from the rearing systems and not been accounted for in the measures of apparent feed intake. Hence, future studies my need to improve the method for measuring lobster AFI.

5.5.2 Food preference

The emergent juvenile of *J. edwardsii* demonstrated greater preference for fresh mussel by ingesting greater amounts of MG followed by MM, and MFD. However, the lobster growth response was not linked to the food preference. This finding provides further evidence that spiny lobsters were more attracted to fresh mussels over artificial diet (Crear et al., 2000 ; Tolomei et al., 2003 ; Dubber et al., 2004 ; Williams et al., 2005 ; Simon & James, 2007). The poorer preference for MFD could probably related to factors such as loss of attractiveness during emersion which can cause exhaustion of leaching nutrient from the feed such as soluble protein, glycine and taurine (Tolomei et al., 2003 ; Williams et al., 2005 ; Williams, 2009). In this study, leaching of the nutrient compound was not measured. Though the leaching rate of MG is greater than MM and MFD, the emergent juveniles displayed a greater preference for MG than MM and MFD. This could be a result of mussel possibly having greater chemoattractant compare to MFD as previous study showed that MG is high in glycine (Williams et al., 2005) which is a common chemical substance found in high concentration in prey tissue which can stimulate specific chemoreceptor neurons in crustaceans (Fuzessery et al., 1978 ; Zimmer-Faust et al., 1984 ; Carr & Derby, 1986 ; Carr, 1988 ; Ache et al., 1988) and increase feed intake of *J. edwardsii* (Sheppard et al., 2002). Leaching of nutrient could also affect the nutrient composition of the feed and result in a suboptimal nutritional profile

(Tolomei et al., 2003). Previously, Ward et al., 2002 explained that the relative growth response of *J. edwardsii* juveniles could confidently be associated with the nutrient composition of the diets where growth rate increased with increasing dietary protein to a maximum. Hence, the absence of correlation observed between food preference and growth may possibly related to differences in the nutritional composition as well as other factors such digestibility and nutrient assimilation of the diet (Williams, 2009 ; Perera & Simon, 2015). Further experimental investigations are needed to examine the feed energy and nutritional values, digestibility, absorption and conversion, and evaluate the effect of feed immersion time on feed intake, growth, food conversion and nutrient leaching.

5.5.4 Conclusion

The result of present study demonstrated that individual feed intake of emergent juvenile *Jasus edwardsii* is not affected by metabolic phenotype. Furthermore, result also demonstrated that growth performance of the juvenile is not linked with their feed intake. The juvenile lobster displayed higher food preference on mussel gonad. However, food preference is not linked with individual growth performance. Overall, these findings show that spiny lobsters feed intake and food preference are not the important factors linking the relationship between metabolic phenotype and growth performance suggesting that mechanism underpinning between metabolic phenotype and growth is intricate and may involve a range of intrinsic and extrinsic factors which need to be explored.

CHAPTER 6

GENERAL DISCUSSION

6.1 Findings of the present study

The present study is the first attempt at understanding how intraspecific diversity in physiological traits, behaviour and feeding can influence the growth performance of two commercial temperate spiny lobsters species, *Sagmariasus verreauxi* and *Jasus edwardsii* juveniles in captivity. This chapter aims to discuss the significant findings of this study and their implications, deliberate on the limitations of the research and highlight future research.

Intraspecific variation in metabolic rate; standard metabolic rate (SMR), routine metabolic rate (RMR), active metabolic rate (AMR) and aerobic scope (AS), collectively known as metabolic phenotypes had a measurable influence on spiny lobsters growth performance when social interaction was absent and food was in abundance. Experiments with *S. verreauxi* early juveniles and, *J. edwardsii* emergent juveniles revealed that differences in growth in both species could be influenced by individual variation in metabolic phenotypes with higher metabolic phenotypes having greater growth performance than lower metabolic phenotypes (Chapters 2 and 4). This result is the first study, to my knowledge, to explicitly describe a link between inidviduals variation in metabolic phenotypes and growth performance of crustaceans. The result also compares favorably with previous studies on teleosts and further supports the important role of metabolic physiology in determining individual aquatic animal growth (Metcalfe et al., 1995 ; Millidine et al., 2009 ; Auer et al., 2015b ; Metcalfe et al., 2016 ; Killen et al., 2017 ; McCarthy et al., 1993 ; Carter et al., 2001). Moreover, this finding also suggests that metabolic physiology is a fundamental factor determining growth in both hatchery-produced and wild stock juvenile spiny lobster culture.

Previously, findings on teleosts suggested that the relationship between energy metabolism and growth are influenced by food availability and intake (Burton et al., 2011 ; Auer et al., 2015a ; Auer et al., 2015c). In contrast in spiny lobster, my findings suggest that metabolic phenotypes were not linked to individual lobster feed intake (Chapters 2 and 5). Additionally, findings in Chapter 5 also demonstrated that lobster growth performance was not correlated with their feed intake. In agreement with earlier studies, *Jasus edwardsii* emergent juveniles demonstrated a preference for mussel notably mussel gonad over a formulated feed. However, food preference was not linked with the individual growth performance. Overall, these findings suggested that in lobsters neither food preference nor feed intake are fundamental factors linking metabolic phenotypes and growth performance. Hence, better growth performance demonstrated by higher metabolic phenotypes lobster reared individually in Chapters 2 and 4 could be possibly due to better feeding assimilation efficiency, nutrient

intake or influenced by other factors such as individual digestive and anabolic capacity as shown in fish by McCarthy (1994) and Allen et al. (2016).

Spiny lobsters are known as highly gregarious social species and previous studies have documented that spiny lobsters often aggregate with their conspecific in shelters (Berrill, 1976 ; Butler & Herrnkind, 2000 ; Childress et al., 2007). In the current study, the influence of social interaction on spiny lobster growth performance was investigated (Chapters 2 and 4). Findings showed that social behaviour induced a strong influence on *S. verreauxi* and *J. edwardsii* growth and also influenced growth disparity and depensation in populations. Moreover, results demonstrated that social interaction outweighed the direct link between metabolic rate and lobster growth signifying that social behaviour plays a more dominant role in determining the growth of individuals in a population. Both species studied showed that lobster feed intake increased and growth performance improved with the influence of social interaction. These findings are consistent with previous results on *Panulirus ornatus* (Irvin & Williams, 2008 ; Ratunil Jr, 2017) and *Panulirus cygnus* (Vijayakumaran et al., 2010) and further support the suggestion that communally reared lobsters display greater growth performance compared to individually reared lobster which is an important consideration for commercial aquaculture. The lower survival of *Panulirus ornatus* in communal culture may be compromised by cannibalism (Irvin & Williams, 2008 ; Ratunil Jr, 2017) but in the present study, survival shown by *S. verreauxi* and *J. edwardsii* was high with no indications of cannibalism. The results could possibly be linked to differences in the social behaviour of *S. verreauxi* and *J. edwardsii*. *Sagmariasus verreauxi* and *J. edwardsii* were formerly included in the same genus *Jasus* before *S. verreauxi* was separated into a older monotypic genus *Sagmariasus* (Holthuis, 1991). Both species are known to be highly gregarious (Butler et al., 1999 ; James et al., 2001) in comparison to *Panulirus ornatus* which has been reported as more solitary and aggressive species, particularly during early juvenile development (Childress et al., 2007 ; Irvin & Williams, 2008 ; Dennis et al., 1997). Suggesting that if growth rates can be improved then Australian temperate species may be easier to culture to market size than tropical species. Increased feed intake in the current study also suggested that social interaction may provide triggers or cues that stimulate juvenile feeding, possibly in response to competitive interactions (Karplus, 2005 ; Irvin & Williams, 2008). A further assessment in Chapters 2 and 4 showed that metabolic phenotype was not linked to lobster growth performance in communal culture. The present findings are in contradiction with some earlier studies on teleosts where individuals with higher metabolic phenotypes were found to have

more rapid growth due greater potential to process food (Van Dijk et al., 2002 ; Hou et al., 2008 ; Millidine et al., 2009 ; Biro & Stamps, 2010 ; Killen et al., 2016). Contrary findings between spiny lobsters and teleosts could possibly be because of dissimilarities in their social behaviour characteristics.

Although social interaction can be a benefit for spiny lobsters by promoting growth performance, there is also a potential cost to aggregation such as feeding competition which can generate variation in individual lobster growth rates leading to individual growth disparity and population depensation. In the current study, factors influencing behavioural interactions between individuals within populations were examined in Chapter 3. Using a pair-feeding contest behavioural study, the social status (dominant, neutral and subordinate) of early juvenile *S. verreauxi* was investigated by examining the influence of metabolic phenotypes, body size, sex, feeding contest experience and rearing history. Upon introduction of food, dominant lobsters had more aggressive behaviour than subordinate lobsters. As would be expected larger sized lobsters were more dominant than smaller sized lobsters (Ranta & Lindström, 1992 ; Smith et al., 1994 ; Thomas et al., 2003 ; Briones-Fourzán et al., 2014). More significant was the finding that low metabolic rate lobsters demonstrated greater ability to win over high metabolic rate lobsters. This finding is in contrast to previous results reported on *Macrobrachium rosenbergii* which again is possibly due to different social behaviour characteristics between gregarious spiny lobsters and solitary freshwater prawns (Brown et al., 2003). The greater dominance of low metabolic rate lobsters may also explain why growth was not positively linked with metabolic phenotypes in communal culture as shown in Chapter 2. Furthermore, female lobsters were more dominant than male lobsters irrespective of size and metabolic phenotypes status. Consequently, these findings suggest that dominance behaviour of *S. verreauxi* is complex and that a range of factors including body size, metabolic status and sex can influence dominance status and growth performance of individual lobster in captivity.

6.2 Limitations of the research

While the research aims of my study have been achieved, there were some unavoidable limitations and results which need careful interpretation. One of the major limitations of this research was the time required to measure the oxygen consumption rate ($\dot{M}O_2$) of individual lobsters, which restricted the number of individual lobster replications that could be used and measured in this research. The automated intermittent flow-through respirometry system used to measure the $\dot{M}O_2$ of juvenile lobsters employed a highly rigorous and detailed protocol which

facilitated very accurate and repeatable measurements of respiration over extended periods allowing for an advanced investigation of lobster metabolism (Fitzgibbon, 2010 ; Jensen et al., 2013a ; Jensen et al., 2013b ; Fitzgibbon et al., 2014 ; Fitzgibbon et al., 2017). Approximately 48 hours were required to measure the $\dot{M}o^2$ of each individual lobster which included a 24 h starvation period followed by an 18 h adaption phase and $\dot{M}o^2$ measurement and 2 h to measure background respiration. Consequently, a maximum of only three or four lobsters could be investigated at any one time and restricted the number of animal replicates. This restriction was particularly pertinent in Chapter 3 where experiment was combined with recording a long period of behavioural contest experiment (approximately four days of behavioural experiment observation for each pair of lobster).

As noted, above successful culture of lobsters has only recently been achieved and there was some limitation in the availability of experimental animals, particularly the use of hatchery reared stock. Hatchery produced *S. verreauxi* juveniles were used in Chapters 2 and 3, and for this, the number of early juvenile lobsters available was limited to 40 lobsters. Lobster larval culture is highly intensive and costly requiring over 220 days for *S. verreauxi,* and there was a high demand from other research projects. While this limitation restricted availability to increase the number of experimental animals, it allows the unique opportunity to study the growth performance of hatchery reared juvenile lobsters that to my knowledge is not available at any other institution in the world. Furthermore, the current study has also provided an initial insight into examining the relationship between phenotypic traits and growth performance of lobsters from known genetic pools. In Chapters 4 and 5, wild emergent juvenile *J. edwardsii* were used as an alternative to hatchery-produced juveniles because of the long planktonic larval phase which makes this species more challenging to culture. However, only 60 post-puerulus were available to be used in this study from three wild collections.

6.3 Recommendations for future research

The current study presents a solid base for future research into the relationship between physiology, behaviour and feeding on lobster growth performance in culture. Prior to my research, very little information was available on the mechanism of how physiology, behaviour and feeding traits of individual lobsters could influence growth disparity in spiny lobster culture. The research was carried out on two highly gregarious spiny lobster species; *S. verreauxi* and *J. edwardsii* with similar findings recorded for both species. It presently remains unclear if the findings apply to all temperate spiny lobster species or indeed all species in

general. Further research is required to examine other lobster species, particularly with more solitary and aggressive species, such as *P. ornatus.* Improved growth in communal culture may be offset by cannibalism with more aggressive lobster species and thus be a trade-off among growth rate, growth disparity and survival (Briones-Fourzán et al., 2014).

The present study has delivered the important insight into the effect of phenotype variation (metabolic rate, body size and behaviour) on individual lobster growth performance where results demonstrated that spiny lobster growth can be directly linked to metabolic phenotype with social behaviour appearing to play a greater role in promoting the growth of individuals and populations. More interesting extension to the present research would be to examine the influence of genotype on the individual growth performance (i.e., the influence of fast-growing genotype on the individual phenotype which could affect their growth performance). It is known that phenotype is a result of the expression of an individual animals genotype, as well as the influence of environmental factors and the interactions between genotype and environmental factors. However, in this present study, the influence of environmental factors can be excluded as the lobsters were reared in the same environmental conditions. Furthermore, it is difficult to relate phenotype differences to genotype. The distinction between genotype and phenotype is important in evolutionary theory, where the survival and mating of an organism depends on their traits (phenotype), but it is the DNA (genotype), which is thought to be unaffected by the development of the traits over the life course, and which will be transferred to the next generation. Thus, future studies investigating the influence of genotype-phenotype on individual lobster growth performance may be useful to improve understanding and knowledge of spiny lobster growth and, for the development of spiny lobsters selective breeding management strategies as selection programmes based on combination of genotype and phenotype merit are shown to be most efficient.

Another interesting extension to the research would be to investigate intra-individual variation (Carter et al., 1998) and determine the repeatability of spiny lobster metabolic phenotypes and investigating how it can influence lobster growth performance over time and different stages in production. Repeatability studies on fish and crustaceans have found that metabolic phenotypes are highly repeatable (Maciak & Konarzewski, 2010 ; McCarthy, 2000 ; Huuskonen et al., 2014 ; Auer et al., 2016) but gradually decline across the medium term (10 weeks) and completely disappear in longer terms (15 weeks) (Norin & Malte, 2011). Additionally, research could be conducted to examine the influence of lobster rearing conditions including, social interaction and social hierarchy development on the metabolic

phenotype of individuals. This information may be useful to understand and evaluate spiny lobster growth in culture over a longer period of time.

The feed preference of lobsters is related to numerous factors including nutrient composition, attractiveness and feed stability. Hence, further research is required to investigate the influence of nutrient composition and the influence of nutrient leaching on the feed intake of lobster over time during immersion. Furthermore, examining the effect of nutrient leaching on feed nutrient composition and how it can influence nutrient intake may be a useful way to help explain the lack of a relationship between food preferences and lobster growth.

One interesting direction for future research is to determine the response of spiny lobsters to the introduction of novel feeds and investigate how it can influence the individual lobster feed intake and dominance hierarchy, which may affect growth of spiny lobsters in communal culture. Previous studies on fish demonstrated that substituting a recognized feed with a novel feed may result in permanent or temporary alterations to groups feed intake and dominance rank which may be effects by several factors such as nutritional requirement, presence of harmful components and unfamiliarity or neophobia (Bromley & Adkinds, 1981; Bres, 1989 ; Perera et al., 1995 ; Refstie et al., 1998 ; Wybourne, 1997; de la Higuera, 2001; Carter et al., 2004).

The absence of a relationship between metabolic phenotype and individual lobster feed intake deserves further investigation, particularly given that spiny lobsters are considered as "messy feeders" where food is handled externally by tearing, pulling and grinding apart the food particles from the food mass prior to ingestion using their mouth part which can result in significant loss of small particles of feed (Barker & Gibson, 1977 ; Zoutendyk, 1988 ; Guillaume & Ceccaldi, 2001). Future research needs to consider how to improve the method for measuring lobster AFI particularly for natural diets such as mussels. For example, using an inert marker such as ytterbium (Cox et al., 2011) and radiography technique (Thomas et al., 2002). However, the usage of radiography has limited value for estimating feed intake of *J. edwardsii* where research is still required to focus on identifying methods that could improve the accuracy and precision of the estimates (Thomas et al., 2002). Additionally, future research needs to focus on investigating the spiny lobster assimilation efficiency, digestive and anabolic capacity particularly for examining the relationship with individual feed intake, metabolic physiology and growth. Previous studies on teleosts have demonstrated that individual conversion efficiency, physiology and anatomical traits and, feed intake are highly correlated

(Trudel et al., 2001 ; Metcalfe, 2015 ; Allen et al., 2016 ; McCarthy et al., 1993 ; Carter et al., 1994.).

Further research is required to determine the mechanisms, such as visual, chemical and/or physical association, which promotes improved growth of spiny lobsters in communal culture. The use of visual and olfactory cues have been demonstrated to influence the dynamics of social interaction in crayfish and lobsters (Bruski & Dunham, 1987 ; Smith & Dunham, 1990 ; Breithaupt & Atema, 1993 ; Schneider et al., 1999). Lobsters have been reported to have the ability to recognise the odour of conspecifics such as chemical stimuli produced during moulting, agonistic and reproductive behaviours (Aiken & Waddy, 1980 ; Waddy & Aiken, 1990). Additionally, gregarious characteristics in spiny lobsters are also facilitated by attraction to chemosensory cues from conspecifics (Childress et al., 2007). Communication between individuals within a population is an important aspect of the behavioural range before, during and after interactions. Research on crayfish, *Orconectes rusticus,* demonstrated that chemical cues from individuals are sufficient to prevent any further interaction from taking place between individuals (Bruski & Dunham, 1987). Future studies on spiny lobsters are required to determine the behavioural and sensory modalities which are responsible for the apparent improved growth in association with social interaction in communal culture.

6.4 Conclusion

The current study has highlighted that the growth of spiny lobsters in culture is highly intricate and affected by a range of intrinsic and extrinsic factors. Collectively, the study findings confirm that social behaviours play a more important role in promoting the growth of individuals and populations than metabolic phenotype. Metabolic physiology displayed a more direct influence on lobster growth performance when social interaction was absent and food in abundance. Feeding competition experiments showed that the dominance status of spiny lobsters during feeding competition can be influenced by lobster body size, metabolic phenotypes and sex. These findings suggest that growth performance of spiny lobsters in culture can be influenced by the link between individual lobster physiological traits and social interactions. Examining the influence of initial individual variation in body size and feed intake in the study suggests that both of these factors are not a major driver for growth disparity in culture. The findings suggest that the development of spiny lobster aquaculture systems, for the two-temperate species examined, may involve a trade-off between systems which promote overall growth at the expense of increases in growth disparity between individuals. The

information from the current study contributes to improving the method and knowledge of spiny lobster rearing systems and management strategies and, provides a better understanding of the influence of physiology, behaviour and feeding on lobster growth performance in culture.

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